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Chemical Effects on Vegetation Detectable in Optical Bands 350–2500 nm

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Contract Scientific Authority: A.A. Faust, DRDC Suffield

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Contract Report

DRDC Suffield CR 2008-234

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Executive Summary

This research team examined the spectral response of individual leaves of three common Canadian plant species (poplar (*Populus deltoides, Populus trichocarpa*), wheat (*Triticum aestivum)*, canola (*Brassica napus*)), which were subjected to fumigation with gaseous phase toxic industrial chemicals and chemicals precursor to chemical warfare agents (e.g. ammonia and sulphur dioxide)(TICs). The larger goal was to develop a remote detection and monitoring capability for hazardous events such as a toxic gas leak. An ideal scenario would involve the release of the toxic chemicals investigated here leaked in the landscape. These agents cause stress and damage to surrounding vegetation the extent of which is dependent on dosage and time of exposure. Our findings at the leaf level suggest that damage can be detectable within 48hrs and should last for an extended period, probably up to a week and thus be possibly detectable from space depending on the repeat pass period of satellites (typically < 2 weeks). The detection tool developed and described in this study relies on the fact that damage is highly non uniform from leaf to leaf and plant to plant as one would expect in a field release thus successful detection in the natural landscape simply requires enough affected area (e.g. image pixels) to capture characteristic data trends for each TIC which should be feasible with <50 pixels (e.g. about 200mx200m for 30m pixels of planned HERO satellite). Such an area would encompass a wide spectrum of stress response from plants to TIC exposure and thus enable their detection. Because we developed a detection methodology from leaf observations there is an important knowledge gap that needs to be addressed using field trials to test if the findings of this study can extend to the detection of leaks in the natural environment. The principal unknown is the effect of varying vegetation canopy structural parameters (e.g. canopy gaps, leaf area) and background properties (litter and soil reflectance) on the specific TIC data trends that were identified. Thus, field trials should first be initiated with simulated TIC releases over closed and mature canopies (e.g. mature crops and conifer or broad leaf forests) since these conditions represent a natural extension of this leaf study. These trials could then be followed by releases over canopies over varied structural properties to test the robustness of the method.

The two objectives of this study were to determine if: 1) vegetation subjected to TICs could be distinguished from background vegetation during varying growth stages (new growth to senescence) and environmental stresses; and, 2) different TICs could be distinguished based on the vegetation spectral response. Treatments were designed to allow quantification of the variation in spectra that might be expected due to environmental, developmental, and stochastic effects on the physiological state of individual plants within each species. All plants were grown in controlled environment chambers at the University of Alberta, using standardized conditions.

The study was broken into two phases: 1) to capture the spectral variability of the various leaf growth stages (news leafs to senescing leaves) observed in each of the three plant types; and 2) subjecting the plants to toxic industrial chemical (TIC) and environmental stresses (e.g. drought). In Phase 1 we determined which leaf growth stage of each species would be subjected to Phase 2 treatments based on spectral variability between growth stages and spectral similarity. In Phase 2, plant species were exposed to the following five industrially relevant gaseous phase TICs: ammonia (NH_3), sulphur dioxide (SO_2), hydrogen sulphide (H_2S), chlorine (Cl_2), and hydrogen cyanide (HCN). The experimental data were analyzed to determine if the various treatments result in specific leaf spectral features related to TICs. The results showed that both

environmental stress and TIC treatments induce similar spectral features inherent to plants that can be related primarily to chlorophyll and water loss. These include pigments in the visible and cellulose, lignin, lipids, starches, and sugars in the short wave infrared. Although no specific spectral features could be tied to individual TICs an analysis of the data using vegetation indices, which focus on key spectral bands associated with chlorophyll, pigments and water content, showed that the TICs and environmental stresses result in diagnostic light reflectance data trends from healthy mature to highly stressed leaves. In addition, further analysis showed that combinations of specific reflectance indices could be used to distinguish NH_3, SO_2, Cl_2 (from everything else) from each other consistently across all three species.

Marked differences in physiological and spectral responses were detected for some combinations of species and TICs. Different responses might be attributed in part to inherent differences in the structure and function of each species (e.g. stomatal density, cuticle thickness, leaf architecture, cell wall composition, growth and respiration rate), or the existence and efficiency of specific metabolic pathways for detoxifying TICs. Whatever their source, the existence of species-specific responses of vegetation to TICs presents both a challenge and an opportunity for regional remote sensing. In this study we were able to discern different spectral trends for NH_3, SO_2, Cl_2 and thus to distinguish these TICs. The trends result from the variable leaf response within plants, between plants and between species and it is expected much of the variability observed within species would be preserved or even enhanced in nature. As such it is encouraging for the possible detection of TIC effects on natural vegetation using airborne/spaceborne imagery. However the variability introduced from species to species that also enhances the TIC spectral trends would be a function of the landscape investigated and encompassed by a given data set (or geographical area) analyzed. The next step should involve a field trial.

Table of contents

List of Figures

List of Tables

Chemical Effects on Vegetation Detectable in Optical Band 350-2500nm
Client Ref. No. W7702-05R073/A

1. PROJECT RESEARCH OBJECTIVE

In the context of evaluating the ability of reflective hyperspectral imaging (350-2500nm) to identify vegetation that has been exposed to specific toxic industrial chemicals (TICs), this research team examined the spectral responses from individual leaves of three Canadian plant species subjected to fumigation with gaseous phase TICs. In addition we examined background spectral responses that included spectral variation related to developmental and environmental effects.

Two questions were assessed using the accumulated experimental data:

1) Can vegetation subjected to TICs be distinguished from background vegetation during varying growth stages (new growth to senescence) and environmental stresses?
2) Can different TICs be distinguished based on the vegetation spectral response?

The selection of toxic industrial chemicals was the subject of a previous report by Derek Peddle entitled "DND requirement for Hero: nuclear, biological and chemical detection capabilities (W7702-05R073/A)". The report indicates that a list of 10 chemical agents incorporating the ones of this study was originally defined based on a recommendation by Jean Marc Theriault (DRDC Val-Cartier) to focus on toxic industrial chemicals and chemicals precursors of chemical agents (e.g. ammonia and sulphur dioxide). The study also recommended an investigation of the effects of the chemicals agents on vegetation rather then attempting to directly detect the agents on vegetation given the perceived short residence time of the agents on vegetation.

2. TARGET SPECIES

We required the subject species of this study to conform to the following criteria.

a) Leaves must be flat and have a contiguous surface area of at least 1.4 cm^2 to facilitate high quality spectral measurement at the leaf level (must fill field of view of contact sensor).
b) Subjects should represent a range of prominent plant groups from the Canadian landscape.
c) Subjects should represent a diversity of potential stress response mechanisms.
d) Subjects should be well-suited to laboratory experiments, e.g. they should be readily available, fast-growing, but small enough to fit within growth and fumigation chambers.
e) Some background information about the stress responses of the subject species should be already known.

Based on these criteria, we selected poplar (*Populus deltoides* x *Populus trichocarpa*), wheat (*Triticum aestivum)*, and canola (*Brassica napus*) as target species. Poplar is distributed throughout both the boreal forest and in the parklands that comprise much of the landscape in Canada. Wheat and canola are the most widely cultivated plants in Canada, and both species have many closely-related wild relatives (i.e. grasses and crucifers) that grow widely throughout Canada and the rest of the world.

3. PLANT TREATMENT PROCEDURES

Treatments are designed to allow quantification of the variation in spectra that might be expected due to environmental, developmental, and stochastic effects on the physiological state of individual plants within each species.

3.1 Plant growth

All plants were grown in controlled environment chambers at the University of Alberta, using standard conditions. Specifically, plants were grown in Metromix 360 soil mixture (Scotts, Marysville, OH) in controlled environment chambers at 24°C with 50% humidity, and at a light intensity of 200 µE (microEinstein) supplied by high output fluorescent bulbs (colour rendering index of 85, colour temperature of 3,500 K) on a 16 h light/8 h dark cycle. Canola (*B. napus* var. Westar) and wheat (*T. aestivum* var. HR 5600) were grown from seed, and poplar (*P. trichocarpa* x *P. deltoides* clone H11-11) was grown from cuttings. Wheat and canola plants were 3-4 weeks post-germination at the time of treatment, and poplar saplings were generally 8 weeks post-cutting. The number of spectra measured per leaf and the number of leaves measured per plant is addressed below in section 4.

3.2 Definition of treatments: Phase 1 & 2

Two phases comprised the design of experiments for spectral analysis. Phase 1 was initiated to capture the spectral variability of the various growth stages (e.g. new leaves to mature leaves to senescence) observed in each of the three plant types. Phase 2 involved subjecting the plants to toxic industrial chemical (TIC) and environmental stresses. Table 1 lists the Phase 1 growth stages investigated and Table 2 lists the Phase 2 experiments. Appendix A includes a table of the complete details of each experiment. The description of environmental stresses and TIC exposure is provided below in section 3.3 and 3.4.

3.3 Environmental stresses

Each species was subjected to treatments representing environmental factors that could influence spectral responses in the field, and which may influence the detection of TICs by reflectance spectroscopy. NaCl treatment was used as a model environmental stress, since it induces ionic stress, as well as osmotic stress responses that are common to drought, freezing, and salinization. We applied acute NaCl stress by drenching roots with a volume of NaCl solution (150 to 400 mM, Table 2) equivalent to at least three times the volume of the pot in which the plant was contained. Each treatment was compared to a mock-treated parallel control. We also imposed drought stress, by with-holding of regular watering from treated plants, while control plants grown in parallel received the normal amount of water. Drought-treated (dehydration experiments listed as H_2O) plants were analyzed spectrally when the soil had fully dried and plants began to show visible signs of stress (e.g. wilting).

Table 1: Phase 1 experiment: growth stages [(1)]

Poplar	Wheat	Canola
G1 Mature: middle to lower 1/3rd	G1 Early growth	G1 New first generation
G2 New growth (top 3 leaves)	G2 Mid growth with stems, no flower	G2 Mature lower stem
G3 Secondary branch leaves	G3 Mid growth with stems, with flower	G3 Mature upper stem
G4 Senescence		G4 New growth
		G6 Senescence

(1) G5 label was used for errors in spectral measurements (e.g. noisy spectra, white reference). This is the same for all three plants species.

Table 2: Phase 2 experiments: environmental stresses and TICs [(1)]

Species	NaCl	H_2O [(2)]	NH_3	Cl_2	SO_2	H_2S	HCN
Poplar	500mM 24 X	24/48	150-200ppm 24				
	150mM 24/48	24	30-40ppm 24				
	150mM 24		variable 24/48				
	150 mM 24/48						
Wheat	200mM 24/48	48/72/96/ 120	150ppm 24/48	<5ppm 24/48	100ppm 24/48		>>50ppm 24/48
	400mM 24/28		50ppm 24/48	15ppm 24/48	0-30 ppm 24/48		
			200ppm 24/48	2-3ppm 24/48	15-20ppm, 24/48		
			140-200ppm 24 X	15-20ppm 24/48 X	75ppm 24/48 X		
				15-25ppm 24/48	60-120ppm 24/48		
Canola	150mM 24/48	24/48	150ppm 24/48	3ppm 24/48	50ppm 24/48	150ppm 24/48	>>50ppm 24/48
	150mM 24 X	48/72/96/ 120	50ppm 24/48	15-20ppm 24/48	100ppm 24/48	300ppm 24/48	
	400mM 24/28		150-200ppm 24/48	<5ppm 24/48	<20ppm 24/48		
			150ppm 24/168 X	15ppm 24/48	40-100ppm 24		
			50ppm24/48	2-3ppm 24/48	30ppm 24 X		
				15-20ppm 24/48 X	15-20ppm 24/48		
				15-25ppm 24/48	75ppm 24/48 X		
					60-120ppm 24/48		

(1) Listed as: dosage, measurement time in hours (approximate).
(2) Dehydration experiments listed as H_2O.

3.4 Application of toxic industrial chemicals (TICs)

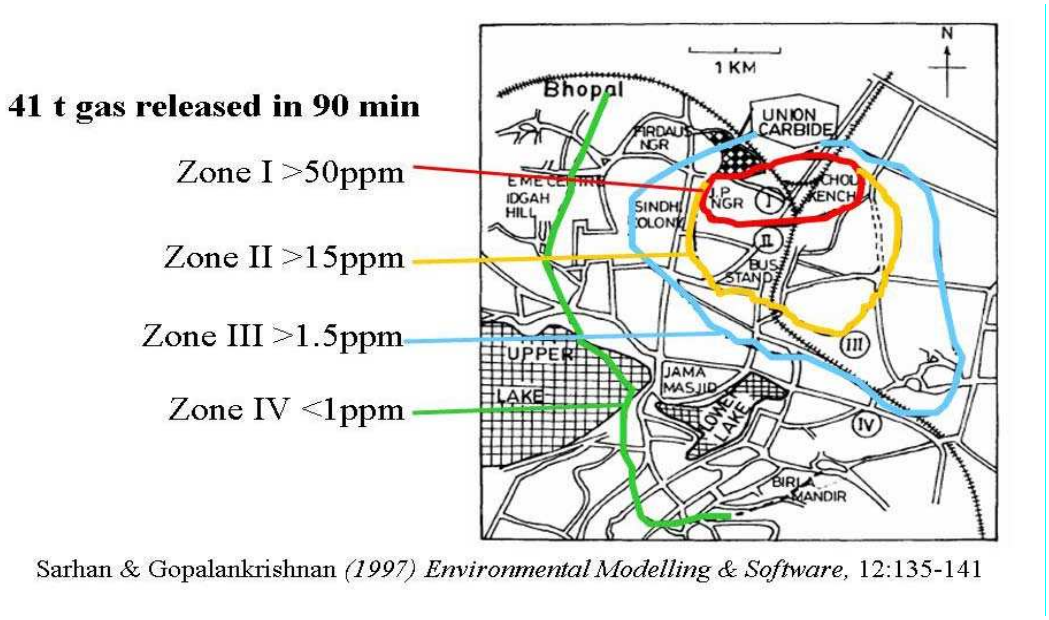
We exposed plant species to the following five TICs: ammonia (NH_3), sulphur dioxide (SO_2), hydrogen sulphide (H_2S), chlorine (Cl_2), and hydrogen cyanide (HCN), at atmospheric concentrations ranging between 0.5 to > 200ppm (Table 2). However, owing to practical limitations, not all species were exposed to all TICs. For exposure we used gaseous phase exposure (i.e. fumigation) of whole plants as the exclusive method of application of TICs, since this is directly relevant to the field situation. Alternative methods of exposure (e.g. painting or spraying) are difficult to replicate and quantify, require solubilization of the TIC in a liquid, and are likely to produce artifacts.

The duration and concentration of exposures were major considerations in the experimental design. We determined to use short-term exposures (i.e. <30 min), because this seemed to best reflect the field conditions that we were trying to mimic, and short-term exposures also minimized confounding experimental effects that might be induced by growing plants for a long period within a plexiglass box. The range of exposure concentrations was determined by several practical and theoretical considerations. For practical reasons, the concentrations needed to be within the range that could be detected by the industrial hygiene monitors described below (0.5-200ppm, depending on the TIC sensor). The concentrations also needed to be higher than might be encountered incidentally under ambient conditions (<5ppm, Table 3), but lower that what might cause an acute safety risk to laboratory personnel. For theoretical reasons, we also considered what concentrations might be encountered within the landscape, in case of TIC release in the field, based on limited reports modeling accidental gas releases such as methyl isocyanate in the Bhopal disaster (Figure 1). Finally, we also adjusted the target treatment concentrations based on the results of the ongoing spectral analysis, to obtain a representative range of responses induced by the treatment concentrations listed in Table 1.

Table 3. Perspectives on incidental and toxic concentrations of TICs.

Gas	Incidental levels (ppm)	REL_{15min} (ppm)	$LC_{50\ 15min}$ (ppm)
NH_3	5 (breath)	35	5000
H_2S	5 (flatulence)	10	>500
SO_2	1 (air)	5	3000
HCN	0.2 (air)	5	100
Cl_2	5 (eye irritation)	0.5	200

REL= recommended exposure limit. LC_{50} = lethal concentration for 50% of a laboratory animal population. Data from WHMIS and related sources.



Sarhan & Gopalankrishnan *(1997) Environmental Modelling & Software,* 12:135-141

Figure 1. Accidental methyl isocyanate gas release from the Bhopal disaster.

Plants were exposed individually to each TIC within a custom built 36cm x 36cm x 75cm plexiglass box, which, during exposures, was covered loosely with a plexiglass lid (Figure 2). Poplar plants were exposed individually, while up to eight wheat and canola plants could be treated simultaneously. Two 11cm, 12V computer fans were used to circulate the atmosphere within the box. TICs (except for HCN, as described below) were introduced into the box through a ¾" (internal diameter) tube, which used a stream of house-compressed air as a carrier. During mock treatments, only the carrier gas was applied to the chamber (without any TICs). NH_3, SO_2, H_2S, and Cl_2 were supplied from compressed gas cylinders (lecture bottles), through a stainless steel regulator (Sigma Aldrich P/N Z148594). Because a commercial mass flow controller that was suitable for corrosive gases ultimately could not be found, the outlet pressure on the regulator was adjusted manually throughout the course of the treatments to try to maintain the concentration of TIC within the treatment box within a target range. We monitored the concentration of TIC within each chamber throughout the duration of the exposures by using a Drager PacIII personal safety monitor that had been fitted with the appropriate sensor for each TIC. In some cases, the concentration of the TIC briefly exceeded the saturation limit of the detector. Although the regulator was thoroughly purged with dry air after each treatment, the regulator needed to be replaced every few months, due to corrosion from the TICs.

Because we were unable to purchase a convenient volume of HCN in a compressed cylinder, we exposed plants to gaseous HCN vapors as follows. Potassium cyanide crystals (KCN) (7.5g for 50ppm) were placed in a 200ml glass beaker with a magnetic stir bar, and the beaker was placed on top of a solid-state magnetic stirrer within the treatment chamber, which also contained the plants for treatment. The lid was placed on the container, while 50% glacial acetic acid (v/v in water) was dripped, with stirring, into the beaker from outside the chamber, through a polypropylene tube, until the target concentration in the chamber atmosphere was obtained.

Figure 2. Plants exposed in custom plexiglass box, which, during exposures, was covered loosely with a plexiglass lid.

4. SPECTRAL DATA AND ANCILLARY DATA

4.1 Data collection

4.1.1 Spectral measurements ASD® Spectrometers

The majority of spectral measurements were acquired using an Analytical Spectral Devices (ASD®) Fieldspec FR spectrometer. This instrument operates from 350 to 2500 nm, where full width half maximum is 3 nm at 700 nm and 10 nm at 1400 and 2100 nm. The sampling interval is 1.4 nm between 350 nm and 1050 nm and 2 nm between 1000 nm and 2500 nm.

Owing to unscheduled maintenance of the FR spectrometer, experiments on March 27-29, 2007, were acquired using the ASD® Fieldspec Hand Held spectrometer. This instrument operates from 350 to 1075 nm, where full width half maximum is 3.5 nm at 700 nm and the sampling interval is 1.6 nm. Data collected from each spectrometer was analyzed, however owing to inconsistencies in wavelength calibration between the two instruments the data cannot be compared directly.

Wavelength calibration for experiments completed May 31st and June 1st, 2007, were offset owing to a technical error in the wavelength calibration file. This data has been wavelength adjusted for each of the three spectrometers within the ASD® FR unit (350-1000, 1001-1830, 1831-2500 nm) and recalibrated to the proper wavelength calibration file. The correction over the 350 to 1000 nm spectral range has an error of +/- ~1nm. There is minimal error over the

1000 to 2500 nm range, except for a flattening of the reflectance spectra at the overlap between the 2nd and 3rd spectrometers.

In some earlier experiments (Phase 1) the ASD® FR spectrometer was experiencing problems over the ~975-1265 nm range, which resulted in a wavy pattern over this range. This pattern did not occur for all spectra. When this pattern did occur, analysis was adjusted accordingly such that results were not adversely affected by this problem. These anomalies did not impact the analysis presented below.

On February 13, 2007 the ASD® FR spectrometer (canola NH3 experiment 4) experienced a problem at the overlap between the 2nd and 3rd spectrometers. The overall effect on the spectral measurements is minimal. However, care was taken in subsequent analysis such that results were not adversely affected by this problem.

4.1.2 ASD® leaf clip attachment

To acquire each spectral measurement on a given leaf the spectrometer is connected to an ASD® leaf clip, which has a fixed viewing and illumination geometry. The leaf clip covers a circular area 10 mm radius using a halogen bulb emitting a colour temperature of 2911 +/- 10°K with specular reflectance not exceeding 5%. Leaf reflectance was calculated by dividing leaf radiance by that of a white reference (99% Spectralon reflectance panel, Labsphere, Inc., North Sutton) measured and illuminated under the same conditions. The white reference is on a reversible platform with the opposite side containing a dark panel, which has an average reflectance of ~0.025%. Figure 3 shows typical reflectance spectra for the white and dark panels. Figure 4 shows the basic set-up for spectral measurements and a zoom of the leaf clip in use. For each poplar and canola measurement a portion of the leaf surface is fully enclosed between the light source and the dark panel. However, owing to the shape of wheat leaves, these leaves only cover a portion of the enclosure between light and dark panel. This results in a reflectance spectrum that is a combination of the leaf and dark panel. This will affect the amplitude, but does not change the shape of the spectrum, which was the focus of our analysis.

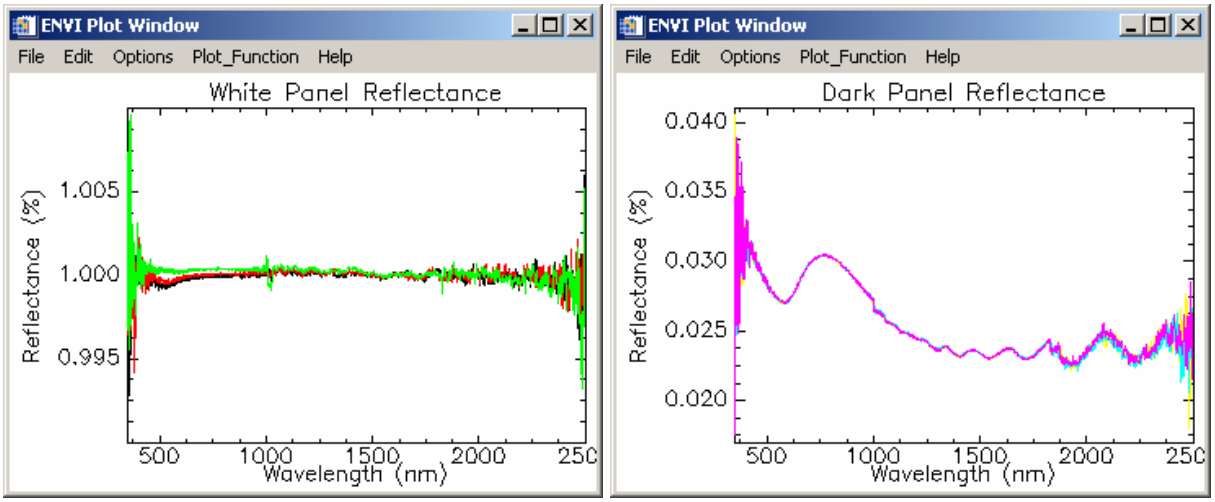


Figure 3. Typical reflectance spectra for the white and dark panels.

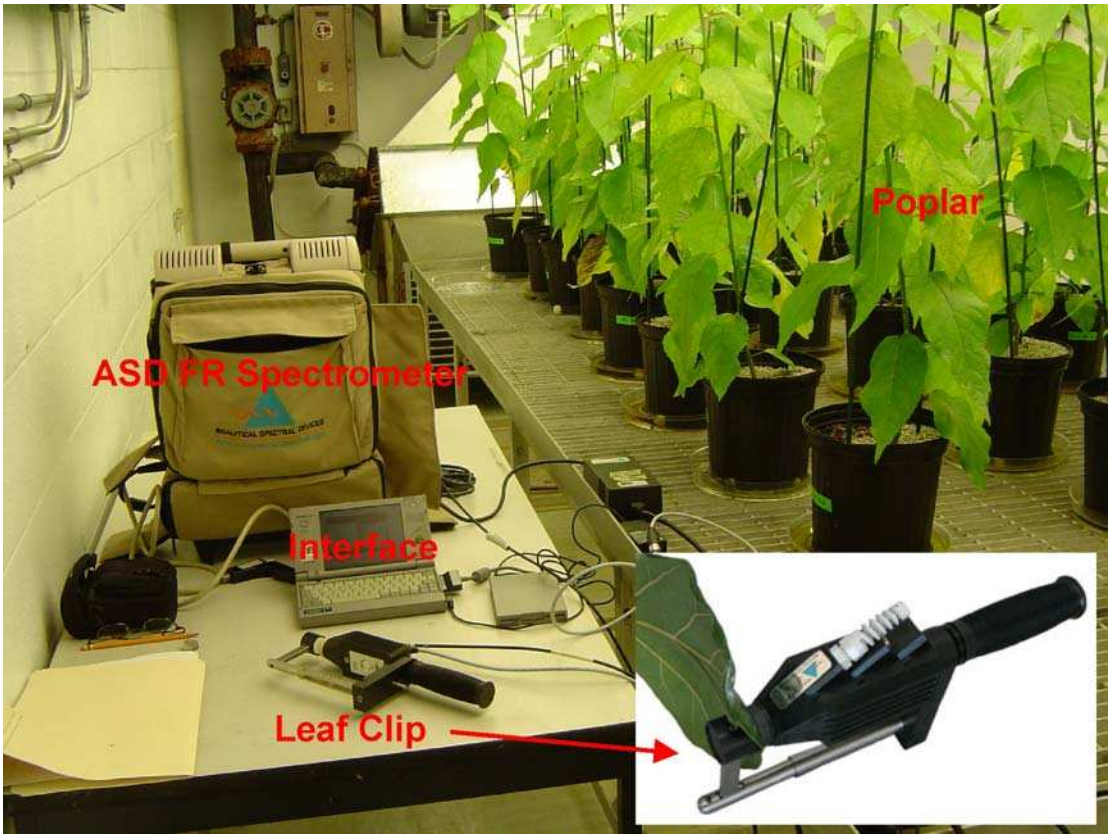


Figure 4. Basic set-up for spectral measurements and zoom of ASD® Leaf Clip in use.

4.1.3 Collection of leaf reflectance spectra

For Phase 1, spectral measurements were taken for 23 poplar, 37 wheat, and 26 canola plants. The number of plants in each Phase 2 experiment varied between 4 and 9, with the majority of experiments including 6 plants per treatment and 6 control plants (e.g. untreated). Generally 3 leaves per growth stage were measured.

Each spectral measurement collected with the ASD® FR spectrometer consists of an average of 10 scans, which takes 1-2 seconds. Multiple scans were taken per leaf location to reduce the effects of noise. For each leaf, three different locations were measured located approximate halfway between the main leaf vein and the leaf edge, precluding overlap of areas measured. The measurements from each leaf were then averaged accounting for spectral variability across the leaf. For smaller leaves (e.g. new growth) only 1 or 2 measurements were possible.

For Phase 2, measurements were taken ~24 hours after treatment and subsequently at 48 hours. In some cases a high degree of stress was visually observed after 24 hours and thus, measurements at 48 hours were not taken. Digital photographs were taken for each plant at both 24 and 48 hours (in a few cases photos were not taken owing to technical problems). Additional photographs were taken of specific leaves showing physiological affects characteristic of a given stress. All data acquired on a given day were recorded on a spreadsheet including: date, plant type, treatment types; and, information relating to each spectral measurement: spectrum number, plant number, treatment type, growth stage, leaf number, digital photographs, and other (e.g. errors). Spreadsheets and digital photographs are included in the HYMEX data delivery Digital Appendix.

4.2 Pre-processing of spectral data

The first step in pre-processing was the conversion from ASD® to ENVI™ spectral library format. All subsequent processing was conducted in the IDL™/ENVI™ environment. Erroneous measurements were then removed, which included experimental errors (e.g. leaf improperly placed in leaf clip), and instrumental errors (e.g. abnormally low signal to noise). Subtle vertical shifts in reflectance can occur at the wavelengths that bound each spectrometer in the ASD® FR unit. These shifts occur because the fibre optic cables connected to each spectrometer do not measure reflectance over exactly to same area of the leaf. Thus, subtle differences in micro topography across the leaf may not be exactly the same for each fibre optic bundle, resulting in the vertical shifts. If a given leaf is uniformly flat, as in the case for the poplar leaves, the vertical shifts are minimal. The shifts are greater for canola, and greatest for wheat leaves. These shifts vary with each spectral measurement. To remove this instrumental shift a constant offset was calculated for the spectral range of each spectrometer and applied to all bands. The corrections are calculated for every spectral measurement. The next pre-processing step was to average all spectral measurements obtained from an individual leaf. Only the averaged spectra were used for subsequent analysis, whereas the original measurements are archived. The last step of pre-processing was the removal of noisy bands below 400 nm and above 2450 nm. Thus, the resulting spectral range for analysis was reduced to 2051 bands from 2151.

4.3 Description of HYMEX Spectral Library Database

4.3.1 Database layout

The HYMEX spectral library database is organized in the following manner (Figure 5): Phase – plant type – treatment type – experiment – spectral library files. For Phase 1 spectral library files (*.lib) and their header files (*.hdr) are named as follows: Phase, plant, and group number (growth stage). All Phase 1 spectral library files end with CTR (e.g control).

Phase 2 spectral library files (*.lib) and their header files (*.hdr) are named as follows: Phase, plant, experiment number, treatment, and time (e.g. 24 or 48 hrs). For example: phase2_wheat_1Cl224.lib with control for the given experiment listed as phase2_wheat_CTR(1Cl224).lib. Note some experiments use the same plants as controls. Table 4 lists all experiments that use the same control plants. For experiments measured using the Hand Held ASD and experiments with a technical error in the wavelength calibration file, HH (Hand Held ASD) and rclb (recalibrated data) have been added to the end of the file name, respectively.

The database also includes a Digital Appendix where spreadsheets and digital photographs for each experiment can be found.

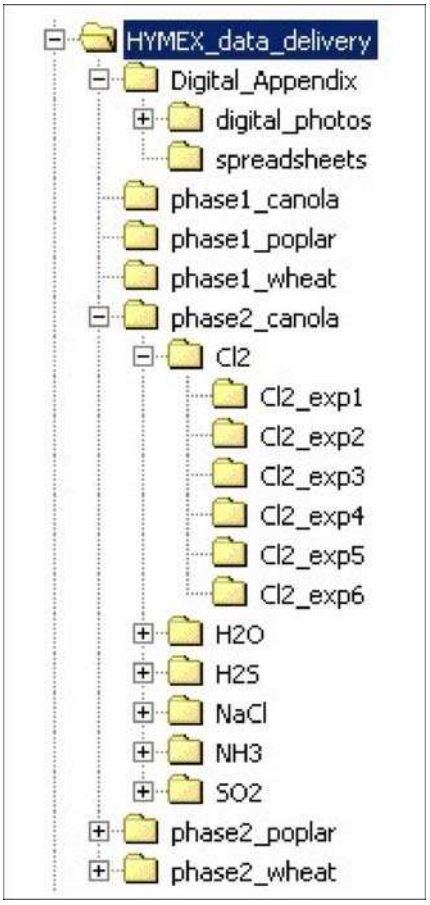


Figure 5. Example of HYMEX spectral library database layout.

Table 4. Experiments that use the same plants as controls[1].

Phase 2 Poplar						
$1H_2O24$[2]						
$1H_2O48$						
$2H_2O$						
1NaCl						
2NaCl24	$1NH_3$					
2NaCl48	$2NH_3$					
3NaCl						
4NaCl24	$3NH_348$					
4NaCl48	$3NH_325$					
Phase 2 Canola						
$1NH_324$						
$1NH_348$						
1NaCl24	$1NH_324$	$2NH_324$	$1Cl_224$	$2Cl_224$	$1H_2S24$	$2H_2S48$
1NaCl48	$1NH_348$	$2NH_348$	$1Cl_248$	$2Cl_248$	$1H_2S48$	$2H_2S25$
2NaCl24	$1SO_224$	$2SO_224$	$1H_2O24$	$3NH_324$		
$1SO_248$	$2SO_248$	$1H_2O48$	$3SO_224$	$3NH_324$		
$3SO_248$	$3NH_348$					
$4NH_3168$						
$4SO_224HH$	$5SO_224HH$					
$3Cl_224HH$	$4Cl_224HH$					
$3Cl_248HH$	$4Cl_248HH$					
$6SO_224rcl$	$7SO_224rcl$	$5Cl_224rcl$	$6Cl_224rcl$			
$6SO_248rcl$	$7SO_248rcl$	$5Cl_248rcl$	$6Cl_248rcl$			
$8SO_224$	$7Cl_224$	3NaCl24	$2H_2O48$			
$8SO_248$	$7Cl_248$	3NaCl48	$2H_2O72$			
1HCN24	$2H_2O96$					
1HCN48	$2H_2O120$					
Phase 2 Wheat						
1NaCl24	$1NH_324$					
1NaCl48	$1NH_348$					
$2NH_324$	$3NH_324$					
$2NH_348$	$3NH_348$					
$1SO_224HH$	$2SO_224HH$					
$1SO_248HH$	$2SO_248HH$	$1Cl_224HH$	$2Cl_224HH$	$4NH_3HH$		
$1Cl_248HH$	$2Cl_248HH$					
$3SO_224rcl$	$4SO_224rcl$	$3Cl_224rcl$	$4Cl_224rcl$			
$3SO_248rcl$	$4SO_248rcl$	$3Cl_248rcl$	$4Cl_248rcl$			
$5SO_224$	$5Cl_224$	2NaCl24	H_2O48			
$5SO_248$	$5Cl_248$	2NaCl48	H_2O72			
1HCN24	H_2O96					
1HCN48	H_2O120					

(1) Listed by row.

(2) The number given at the end refers to the time (approximate) measurements were taken in hours.

4.3.2 Spectrum naming convention

Each spectrum in the HYMEX spectral library database is assigned a name that can be referenced to experiments shown in Table 2, Appendix A, and the spreadsheets in the Digital Appendix. The following is the spectrum naming convention:

plant type(1), date(2), number of averaged spectra + original spectra number(3), plant number(4), leaf(5), leaf growth stage(6), experiment(7)(8)(9)(10).

(1) pop = poplar, wht = wheat, can = canola
(2) given as mmddyy
(3) number of spectra to average for single leaf. The number used refers to the first leaf spectra number of those averaged (e.g. 3a024)
(4) plant number
(5) leaf given as A,B,C ...
(6) Growth stage is followed by L, M, or H this refers to the visible level of stress (subjective visible assessment L-Low;M-Medium;H-High).
(7) given as treatment type (e.g. H_2O, NaCl, SO2 etc)
(8) X= X is added to the end of treatment type (e.g. NaClX) if the leaf was cored for chlorophyll analysis. L= L is added to the end of stress type (e.g. NaClL) if the leaf was measured for leaf area index.
(9) experiment number is added to the front of the treatment type (e.g. 1NaCl) to note which repeat experiment the data was from.
(10) 24 or 48 is added to the end of the treatment type to note 24 hours (or 48 hours, or higher). If no time is given then assumes 24hours. For some experiments (e.g. H_2O) time may be greater than 48 hrs.

For CTR samples the corresponding treatment experiment is listed in brackets (e.g. CTR(1NaCl)). Multiple experiments may use the same control plants.

Example: for average of canola leaf measured on March, 29th, 2007, comprising 3 spectral measurements, starting at spectrum 182, from plant 2, leaf A, leaf growth stage, medium visible stress, experiment number 4, for treatment Cl2, measured at 48 hours.

can_032907_3a182_P2_LA_G3M_4Cl248

An example of a control sample used for this experiment would be labeled as:

can_032907_3a212_P4_LA_G2_CTR(4Cl248)

5. DATA ANALYSIS

5.1 Methodology Phase 1

5.1.1 Spectral angle and Statistics

Using the Phase 1 data (investigation of growth stages) we first aimed to determine guidelines for the measurement of Phase 2 leaves, or in other words which type of leaf to measure during treatment? Thus, we examined the spectral variability for the various growth stages of all 3 plant species (refer to Table 1).

To determine the within-class (species) spectral variability we have made use of spectral angle (θ) as described by Price (1994). The θ value represents the angle between the reference and the sample spectrum, calculated using a vector dot product. This index is amplitude independent due to the denominator (Price, 1994). The spectral angle was calculated for: 1) each pair of spectra within a single growth stage (pair-wise spectral angle); and, 2) between the mean reflectance spectra for each growth stage. The spectral angle was calculated for the following wavelength regions:

- 400 to 2450 nm (VIS, NIR, and SWIR)
- 400 to 716 nm
- 717 to 975 nm
- 976 to 1265 nm
- 1266 to 1770 nm
- 1771 to 2450 nm

The near infrared and the short-wave infrared were divided according to the wavelength limits of detectors for the ASD spectrometer used during data acquisition. Pair-wise spectral angle is calculated in order to determine spectral variability between growth stages, where stages that show minimal spectral variability will allow for better discrimination of treated plants without the possible confusion with natural variability. The spectral angle calculated between mean reflectance spectra from each growth stage will determine spectral similarity between growth stages, such that we can determine which growth stages will be focused on in Phase 2.

5.2 Methodology Phase 2

5.2.1 Spectral significance of experiments

A primary goal of Phase 2 was to determine whether leaves subjected to natural stresses and TICs could be distinguished from healthy leaves (e.g. control) or senescent leaves, and whether the nature of the TICs could be determined from the spectral signature of the leaves. The first step of Phase 2 was to determine which experiments resulted in significant spectral changes with respect to control plants (e.g. for low dosage TIC are there significant changes in reflectance for the stressed leaves?). This was accomplished by calculating the pair-wise spectral angle for all the leaves in control sets for each species over the spectral ranges shown in 5.1.1.

The mean pair-wise spectral angle for each spectral range was recorded and used as a threshold to test for significant spectral changes of the various experiments. If the spectral angle between the mean reflectance spectra for an experiment and the mean reflectance spectra for the control plants exceeds that of the threshold for any spectral range the experiment is considered to contain significant spectral changes and warrants further analysis. All spectral ranges are considered, as significant spectral changes may only occur over a particular spectral range.

5.2.2 Endmember Analysis

For treatment experiments that produced leaves with significant spectral differences the next goal was to determine which leaf spectra captured the most severe response relative to healthy leaves. The premise is that if a given TIC results in a unique spectral feature that can discriminate it from natural stresses and/or other TICs, this feature should be visible in the leaf with the most extreme response. To find extreme responses from each experiment we made use of an endmember search algorithm, where endmembers are considered to be spectral extremes that lie at the vertices of the data volume, or simplex, in n-dimensional space. Figure 6 is an example of data volume for poplar Phase 1 data showing healthy mature, new growth and senescence, which form the endmembers of this system.

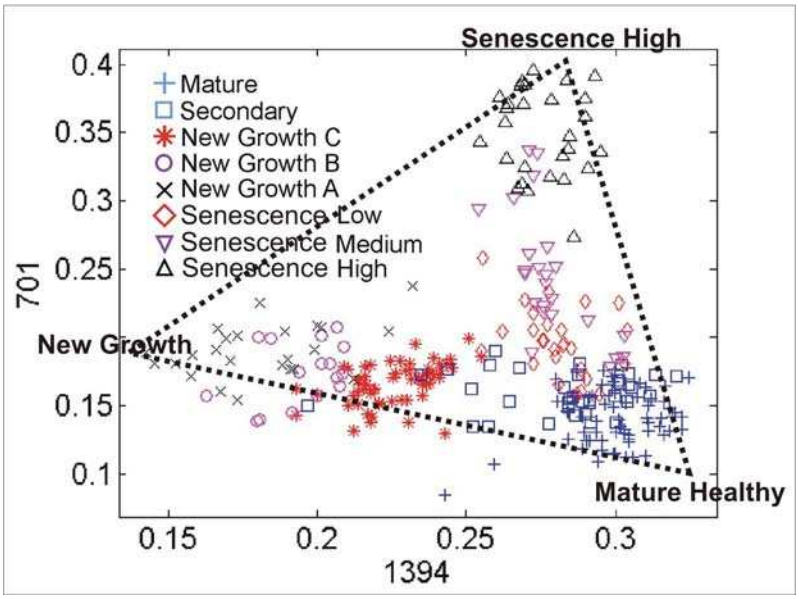


Figure 6. Data volume of poplar data from Phase 1 shown in 2-dimensions (x-axis 1394 nm, y-axis 701 nm). Dotted line represents an approximation of the simplex that defines the data volume encompassed by healthy mature, new growth, and senescence leaves as endmembers. Each point represents the spectral values of a measurement for spectral bands centered at wavelengths 701 nm and 1394nm.

The endmember extraction algorithm is a modified version of the spatial spectral endmember extraction tool (SSEE) (Rogge et al., 2007). Given that the data is structured in the form of spectral libraries rather than images, the spatial component used in SSEE is removed. The SSEE algorithm is completed in n-dimensional spectral vector space and involves two main steps: 1) application of singular value decomposition (SVD) to determine a set of eigenvectors that describe most of the spectral variance for a given spectral library; and, 2) projection of the spectral library onto the eigenvectors and collection of those spectra than lie at either extreme of the vectors. These spectra represent the endmembers of the given spectral library. The resulting endmembers from each experiment are compared with those from other treatments to determine if specific absorption features can be related to a given TIC.

5.2.3 Vegetation Indices

The spectral response observed for treated leaves is highly variable and includes relatively healthy to extreme responses. Thus, the spectral libraries for each treatment include a continuous to semi-continuous representation of the variability of leaf responses, similar to that observed from healthy to senesced leaves seen in Figure 6. The spectral trends from healthy to stressed leaves may then be indicative of a particular environmental stress and/or TIC. For this analysis we chose a number of commonly used vegetation indices that focus on bands related to pigments and water content of leaves. Table 5 lists the indices used, their formulation, the type of index, and related literature. The various indices used include normalizing factors, such that the resulting trends are based on spectral shape and not amplitude.

Table 5. Vegetation Indices used in this study

Vegetation Indices	**Wavelengths given as nanometers (Constants given in BOLD face)**		**Key Reference**
Name	**Index formula**	**Group**	
NDVI	(800-670)/(800+670)	Chlorophyll	Rouse et al, 1974
MCARI1	**1.2**(2.5(800-670)-**1.3**(800-550))	Chlorophyll	Haboudane et al 2004
PRI	(570-531)/(570+531)	Pigments	Gamon et al 1992
PSRI	(680-500)/750	Pigments	Merzlyak, 1999
NPQI	(415-435)/(415+435)	Chlorophyll/Pigments	Barns et al 1992
Red Edge	**700** + **40**((rededge-700)/(740-700))	Red Edge	Guyot and Baret, 1988
	Red edge=(671-781)/**2**		
NDWI	(860-1241)/(860+1241)	Water	Gao, 1996
SWIR1(1)	(2015-2100)/(2015+2100)	Water	

(1) Derived for this study.

6. RESULTS PHASE 1

6.1 Poplar

6.1.1 Spectral differences between poplar growths stages

Spectral measurements were taken from 23 poplar plants, however not all plants had leaves of each growth stage defined in Table 1. Stage G2 (new growth) was divided in 3 subgroups (Figure 7): G2A) large leaves with no petiole (leaf stem) within the field of view (FOV), G2B) small leaves with no petiole in FOV, and G2C) small leaves with petiole in FOV. New growth leaves often had a subtle red coloration and generally did not show the deep green color observed for mature leaves. Senescent leaves (G4) were divided into 3 subgroups: G4A) low senescence, G4B) medium senescence, and G4C) high senescence. These subgroups are based on visual assessment. Figure 7 shows typical examples of poplar leaves at the various growth stages. Table 6 shows the number of leaves per growth stage.

Figure 8 shows the mean pair-wise spectral angle (+/- 1 standard deviation) for each growth stage over the spectral ranges shown in 5.1.1. Figure 9 shows the mean reflectance spectra for each poplar growth stage with the spectral angle between each growth stage mean reflectance spectra shown in Table 7. Included in Figure 9 are key spectral features associated with chlorophyll (A, B), pigments (ß-carotene, pycoerythrin and phycocyanin), cellulose and lignin, and water common to vegetation (from Curran, 1989; Farabee, 1997).

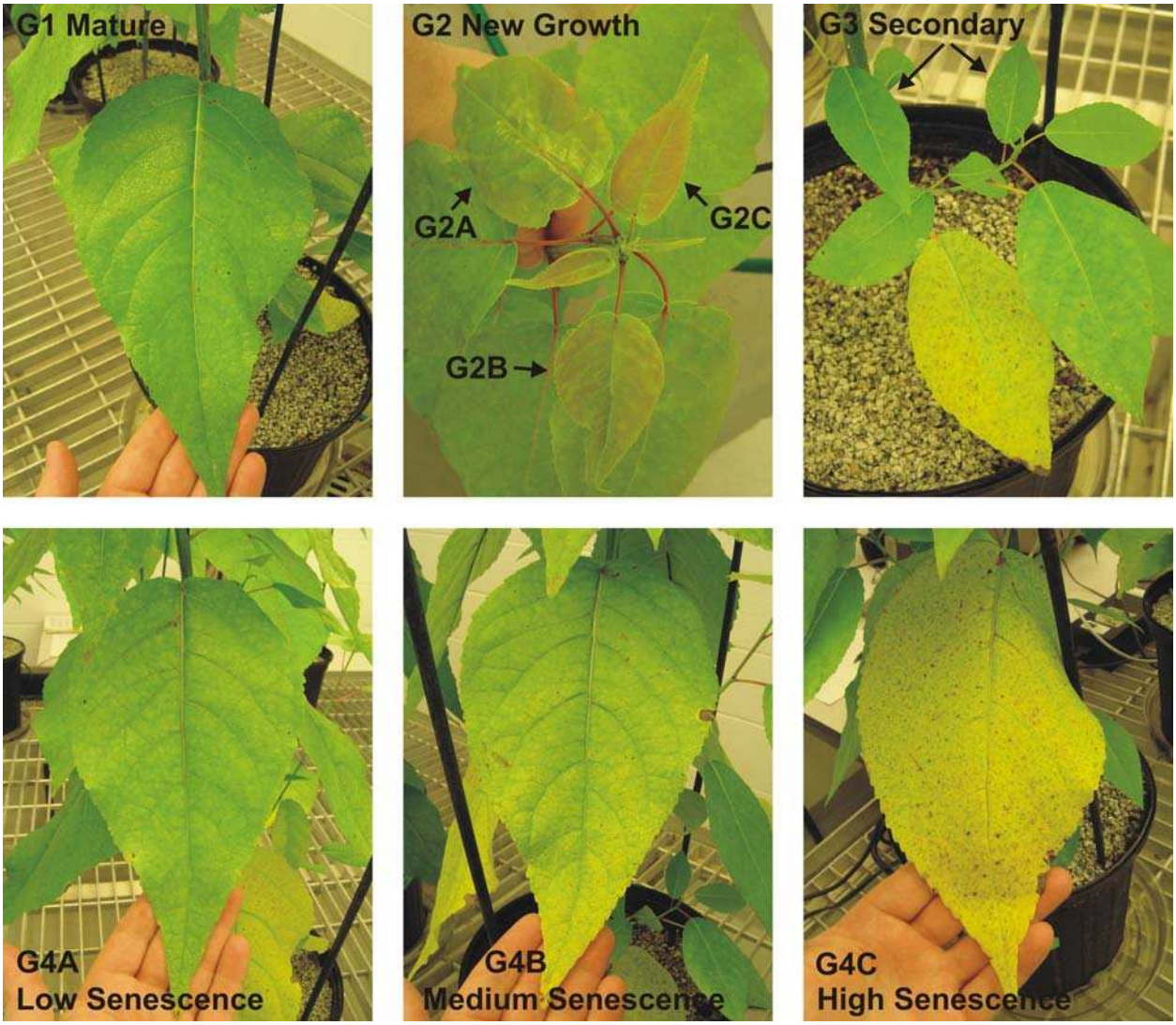


Figure 7. Typical poplar leaves at various growth stages.

Table 6: Number of leaves per poplar growth stage.

	G1	G2A	G2B	G2C	G3	G4A	G4B	G4C
no. leaves	43	40	16	37	72	18	16	19

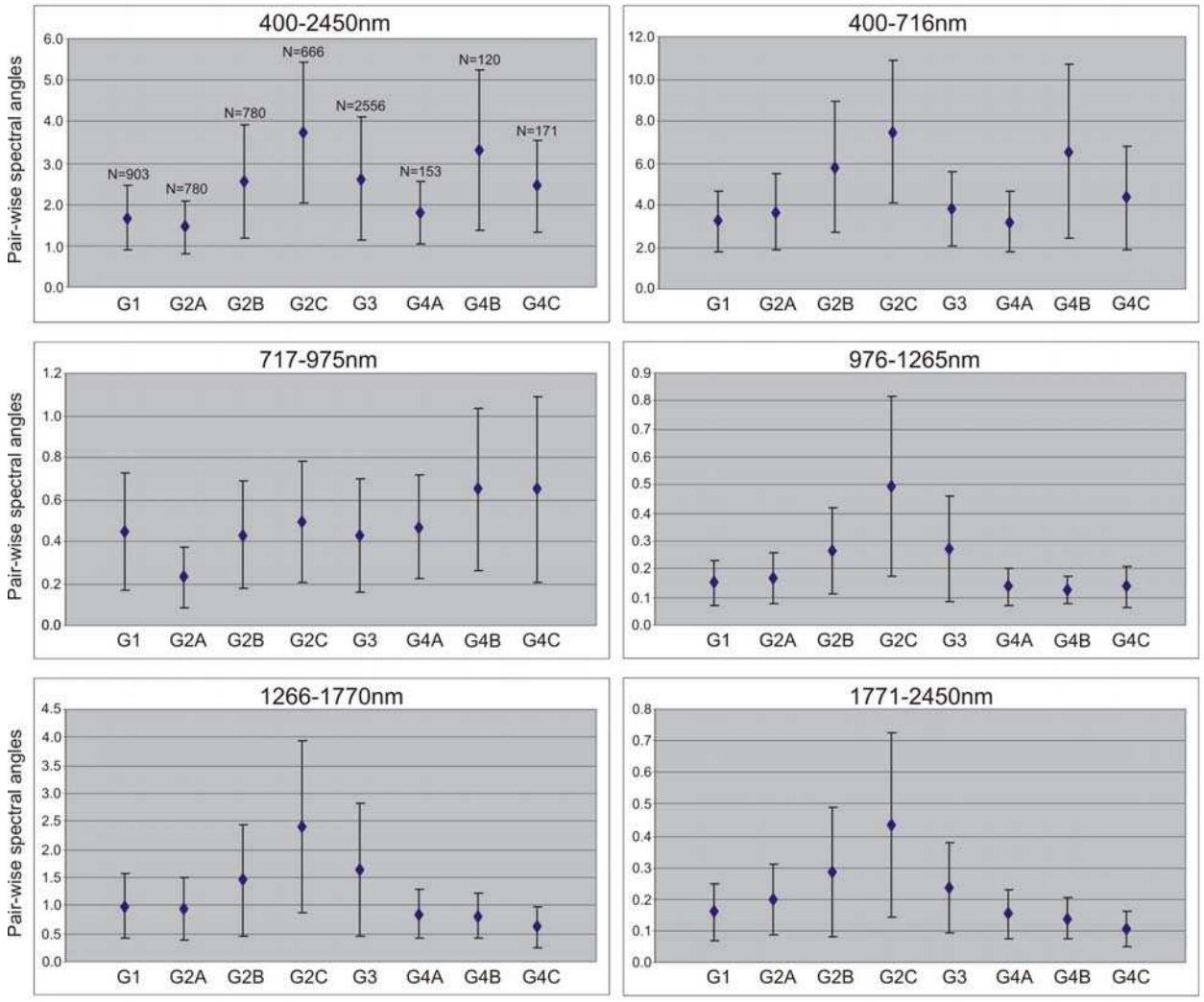


Figure 8. Mean pair-wise spectral angle (+/- 1 standard deviation) of leaf reflectance for each poplar growth stage. Where pair-wise spectral angles are calculated for each pair of leaf spectra within a single growth stage. Number of pair-wise combinations computed for each growth stage is shown in the top left plot.

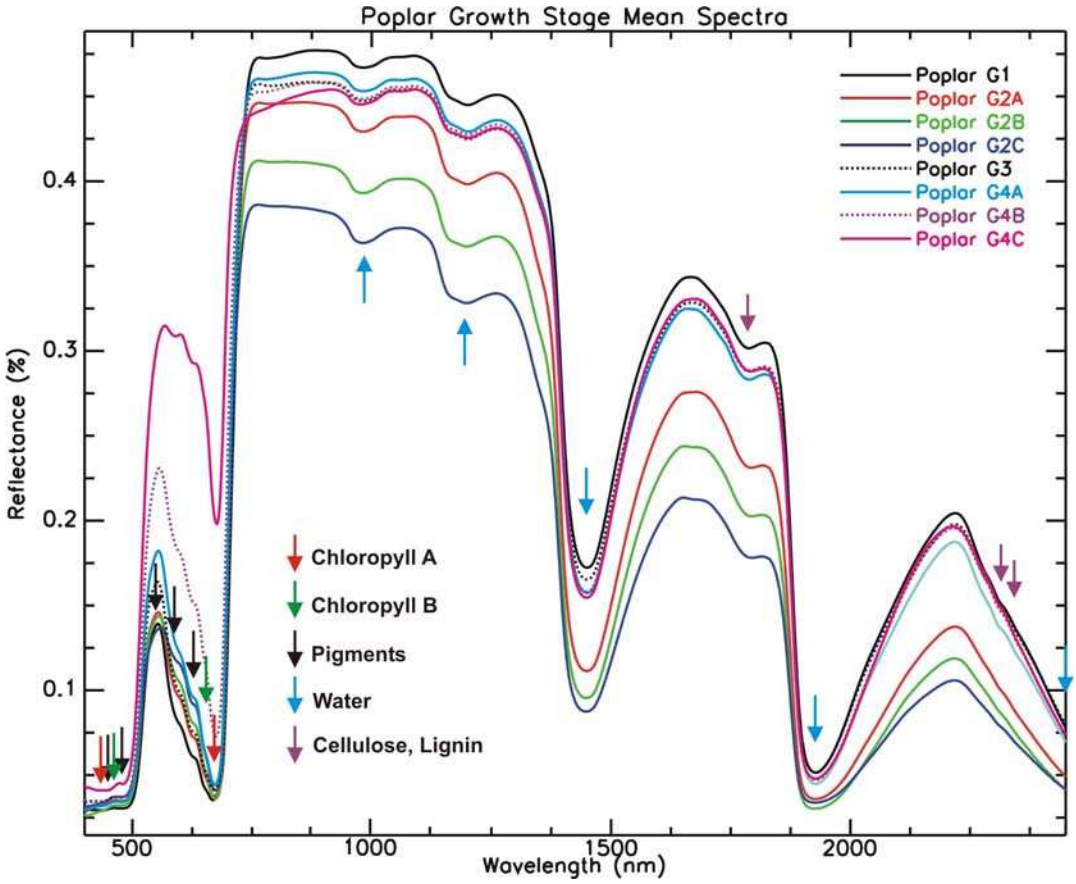


Figure 9. Mean reflectance spectra for each poplar growth stage. Includes key spectral features associated with chlorophyll (A, B), pigments (ß-carotene, pycoerythrin and phycocyanin), cellulose and lignin, and water common to vegetation.

Table 7. Spectral angle (degrees, 400-2450 nm range) between mean spectra of each growth stage shown in Figure 9.

	G1	G2A	G2B	G2C	G3	G4A	G4B	G4C
G1								
G2A	5.99							
G2B	7.37	1.58						
G2C	8.67	3.26	1.85					
G3	**1.55**(1)	5.76	6.95	8.06				
G4A	2.82	4.61	5.62	6.70	1.76			
G4B	5.42	6.53	6.96	7.51	4.07	3.06		
G4C	11.90	11.74	11.46	11.15	10.64	9.66	6.76	

(1) G1 and G3 most similar growth stages based on spectral angle.

6.1.2 Poplar Growth Stages for Phase 2

Mature healthy leaves (G1) showed minimal variability (low standard deviation) when compared to other growth stages (Figure 8) for the spectral ranges shown in 5.1.1.. G3 (secondary leaves) do not always occur in plants and are spectral similarity to G1 based on spectral angle (Table 7). For these reasons they are excluded from Phase 2 experiments. Spectral measurements of G2 (new growth A,B,C) were collected in Phase 2 but considerable spectral variability was observed in this stage over all spectral ranges (Figure 8), thus G2 was not analyzed in Phase 2. Senesced leaves (G4A,B,C) show moderate spectral variability over the 400-975 nm spectral range, but minimal variability beyond 975 nm (Figure 8). Senesced leaves were not measured in Phase 2 because we could not ensure their presence in each experiment. Based on the results shown above for Phase 1 poplar, spectral measurements and analysis for Phase 2 were restricted to mature healthy leaves (G1).

6.2 Canola

6.2.1 Spectral differences between canola growths stages

Spectral measurements were taken from 26 canola plants, however not all plants had leaves of each growth stage defined in Table 1. Growth stages 3 and 4 (mature upper stem, New growth) (Figure 10) show the distinctive blue hue typical of healthy mature canola plants. Growth stage 2 (mature lower stem) leaves are similar to G3 and G4, however, the distinctive blue hue is not as evident. Senesced leaves (G6) were divided into 3 subgroups: G6A) low senescence, G6B) medium senescence, and G6C) high senescence. These subgroups were based on visual assessment. Figure 10 shows typical examples of canola leaves at the various growth stages, excluding senesced leaves. Table 8 shows the number of leaves per growth stage.

Figure 11 shows the mean pair-wise spectral angle (+/- 1 standard deviation) for each growth stage over the spectral ranges shown in 5.1.1.. Figure 12 shows the mean reflectance spectra for each poplar growth stage with the spectral angle between each growth stage mean reflectance spectra shown in Table 9.

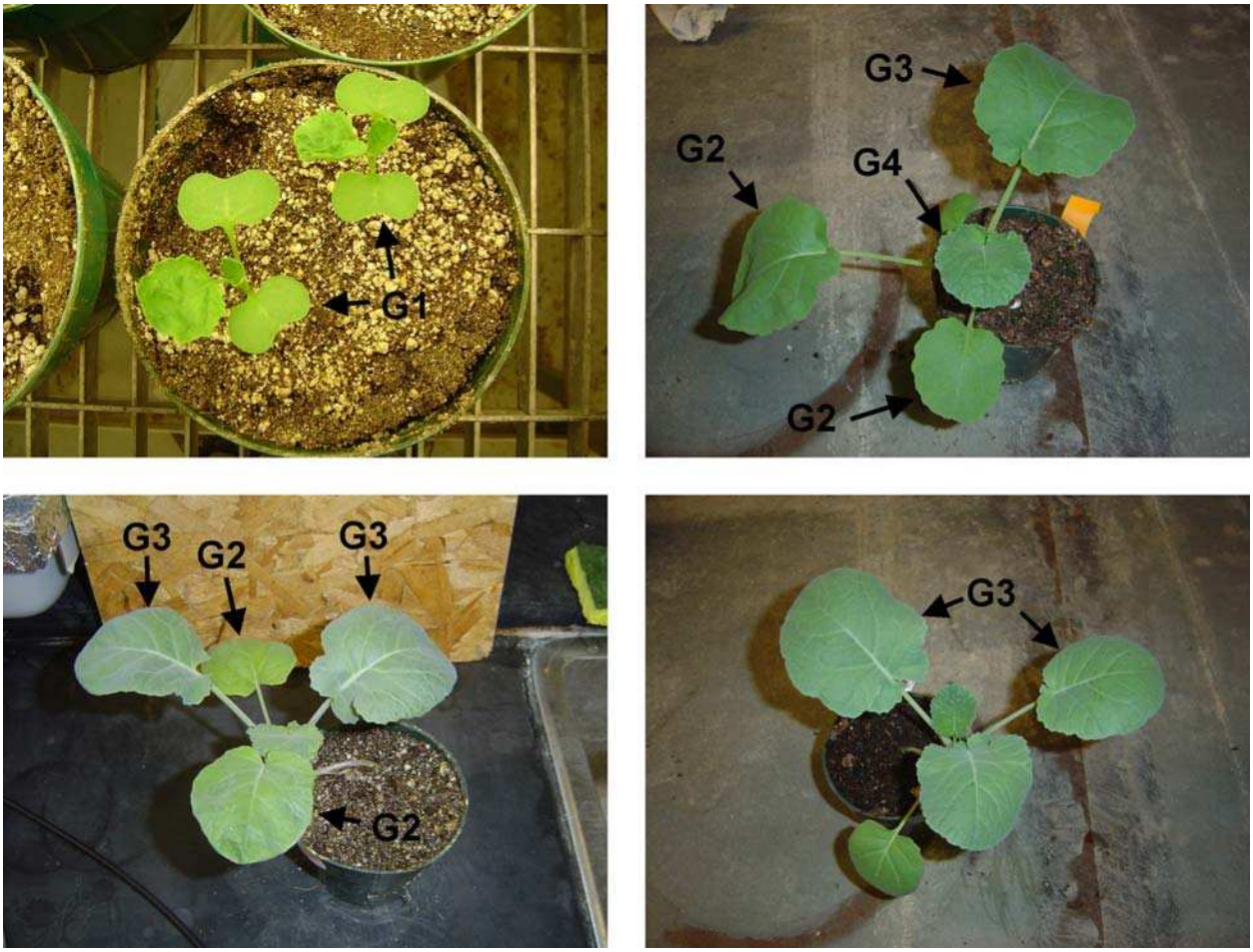


Figure 10. Typical canola leaves at various growth stages (G1: new first generation; G2: mature lower stem; G3: mature upper stem; G4: new growth). Note images of senesced canola leaves not available.

Table 8: Number of leaves per canola growth stage.

	G1	G2	G3	G4	G6A	G6B	G6C
no. leaves	20	23	24	27	5	5	4

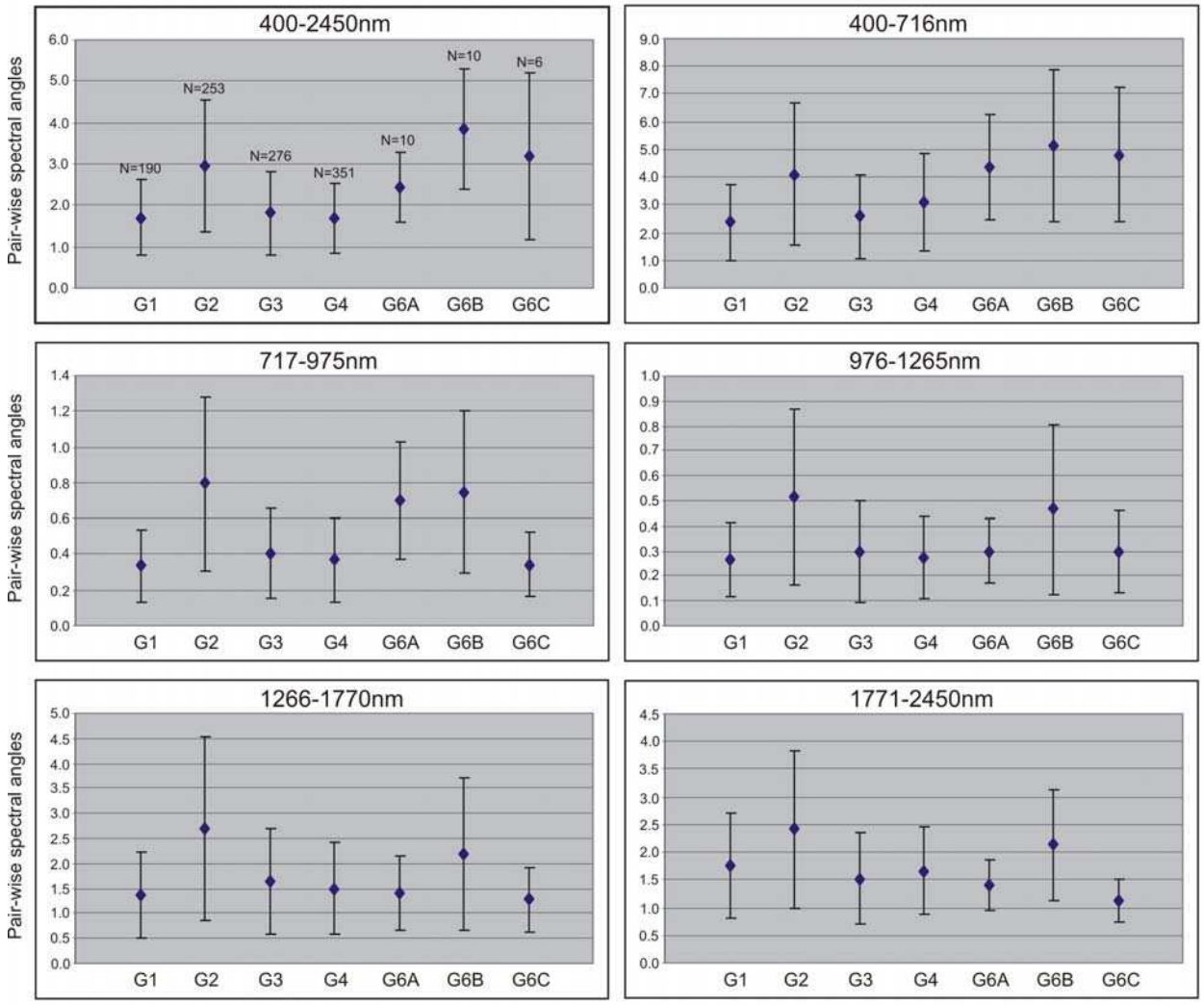


Figure 11. Mean pair-wise spectral angle (+/- 1 standard deviation) of leaf reflectance spectra for each canola growth stage. Where pair-wise spectral angles are calculated for each pair of leaf spectra within a single growth stage. Number of pair-wise combinations computed for each growth stage is shown in the top left plot.

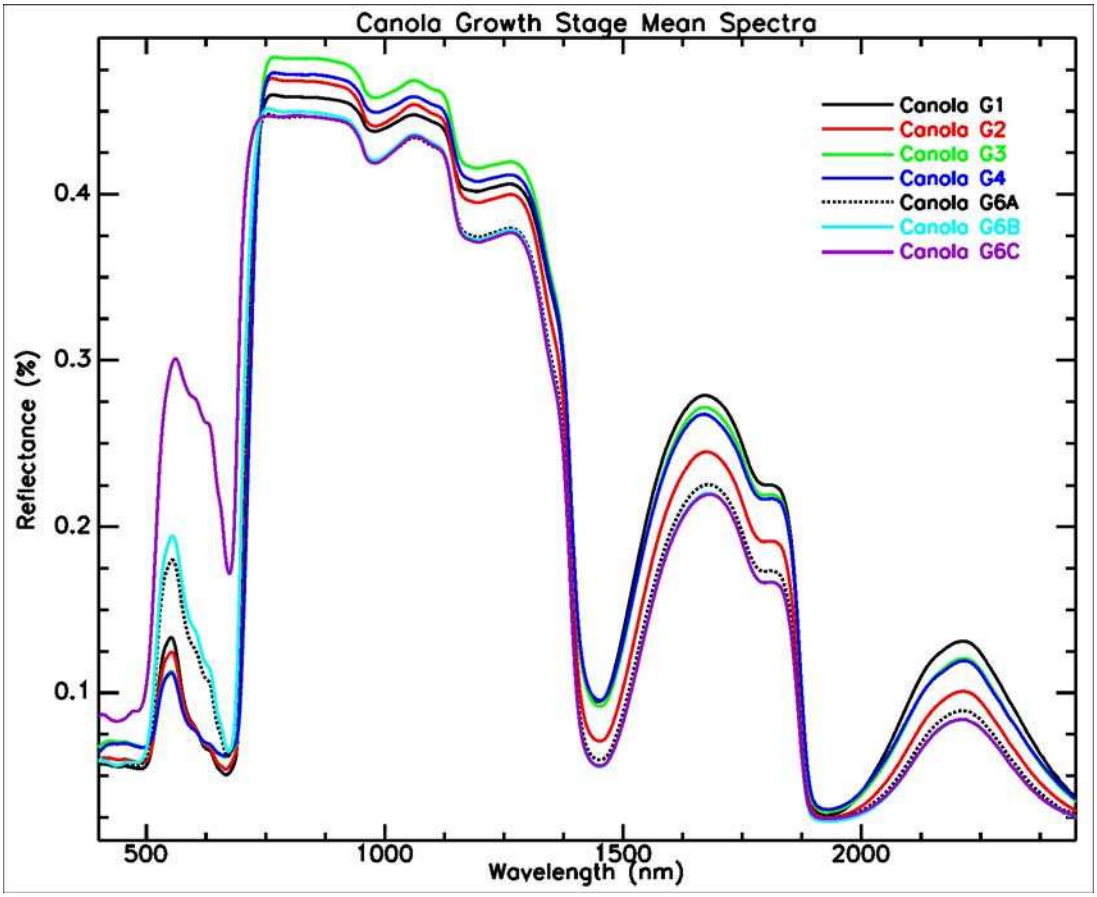


Figure 12. Mean reflectance spectra for each canola growth stage.

Table 9. Spectral angle (degrees, 400-2450nm range) between mean spectra of each growth stage shown in Figure 12.

	G1	G2	G3	G4	G6A	G6B	G6C
G1							
G2	4.16						
G3	2.40	2.59					
G4	2.13	2.86	**0.48**(1)				
G6A	6.43	4.09	5.97	6.03			
G6B	7.52	5.03	7.01	7.09	1.12		
G6C	13.98	12.85	13.99	13.94	9.21	8.50	

(1) G3 and G4 most similar growth stages based on spectral angle.

6.2.2 Canola Growth Stages for Phase 2

Growth stages G1 (first generation), G3 (mature upper stem), and G4 (new growth) show minimal spectral variance (low standard deviation) compared to growth stages G2 (mature lower stem) and G6 (senescence) (Figure 11) over the spectral ranges shown in 5.1.1. G3 and G4 are spectrally similar with a spectral angle of only 0.48 degrees (Table 9). The main difference between these two growth stages appears to be leaf size. Spectral measurements of G2 were collected in Phase 2, but considerable spectral variability was observed in this stage over all spectral ranges (Figure 11), thus G2 was not analyzed in Phase 2. Although G1 leaves show low spectral variability they represent very immature canola and were not considered in Phase 2. Senesced leaves were not measured in Phase 2 because we could not ensure their presence in each experiment. Based on the results show above for Phase 1 canola, spectral measurements and analysis for Phase 2 was restricted to G3 and G4. However, owing to their spectral similarity the two were analyzed together.

6.3 Wheat

6.3.1 Spectral differences between wheat growths stages

Spectral measurements were taken from 46 wheat plants, however not all plants had leaves of each growth stage defined in Table 1. Figure 13 shows typical examples of wheat leaves at the various growth stages. Table 10 shows the number of leaves per growth stage.

Figure 14 shows the mean pair-wise spectral angle (+/- 1 standard deviation) for each growth stage over the spectral ranges shown in 5.1.1.. Figure 15 shows the mean reflectance spectra for each wheat growth stage with the spectral angle between each growth stage mean reflectance spectra shown in Table 11.

6.3.2 Wheat Growth Stages for Phase 2

Growth stages G1 (early growth), G2 (mid growth with stems, no flower), and G3 (mid growth with stems, with flower) show similar spectral variance over the spectral ranges shown in 5.1.1. (Figure 14). G1 and G2 are spectrally similar with a spectral angle of 1.00 degrees (Table 11). Based on the results show above for Phase 1 wheat, spectral measurements and analysis for Phase 2 was restricted to G2. G1 is considered to be spectrally similar to G2 and was excluded in Phase 2.

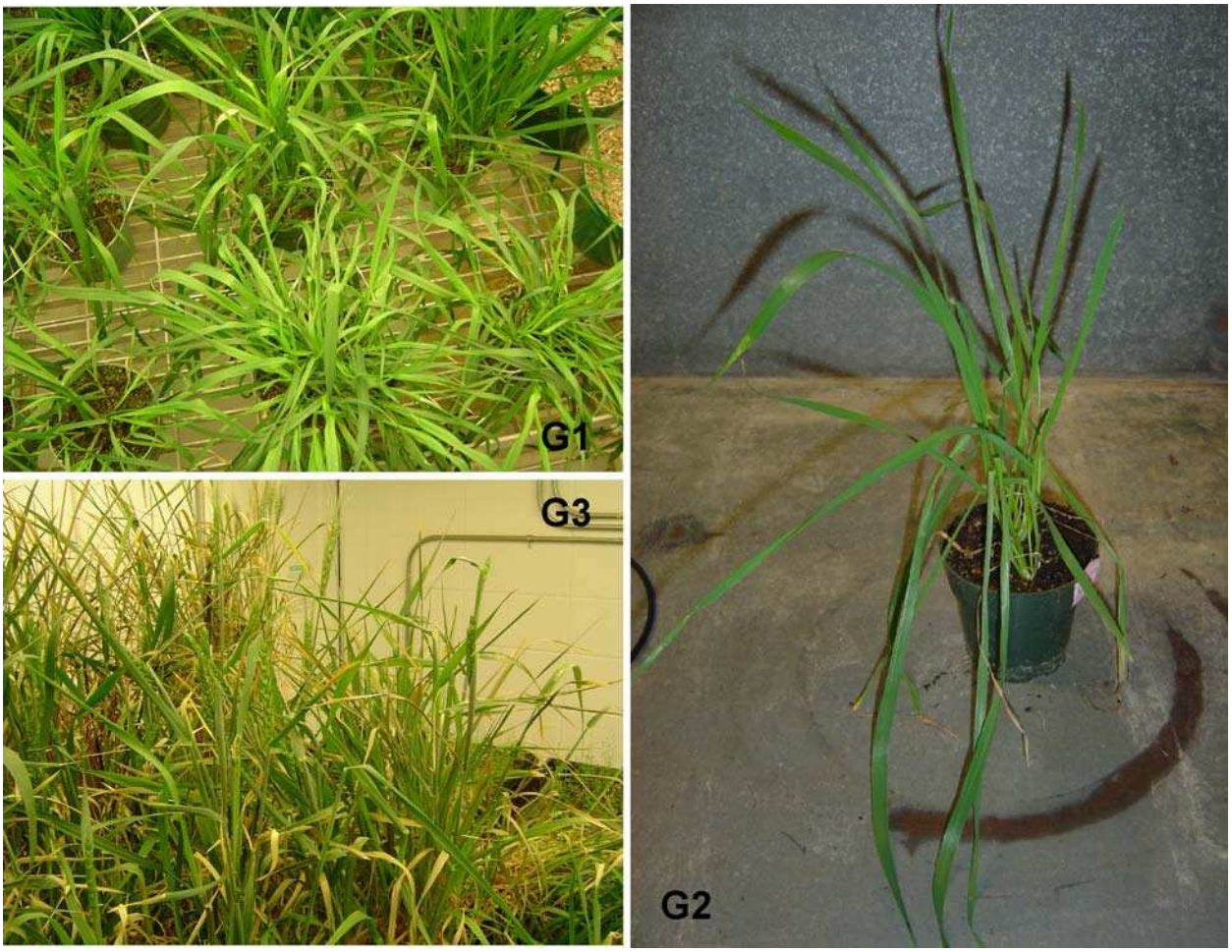


Figure 13. Typical wheat plants at various growth stages (G1: early growth; G2: Mid growth with stems, no flower; G3 Mid growth with stems, with flower). G2 example is typical of plants used in further experiments.

Table 10: Number of leaves per wheat growth stage.

	G1	G2	G3
no. leaves	115	36	24

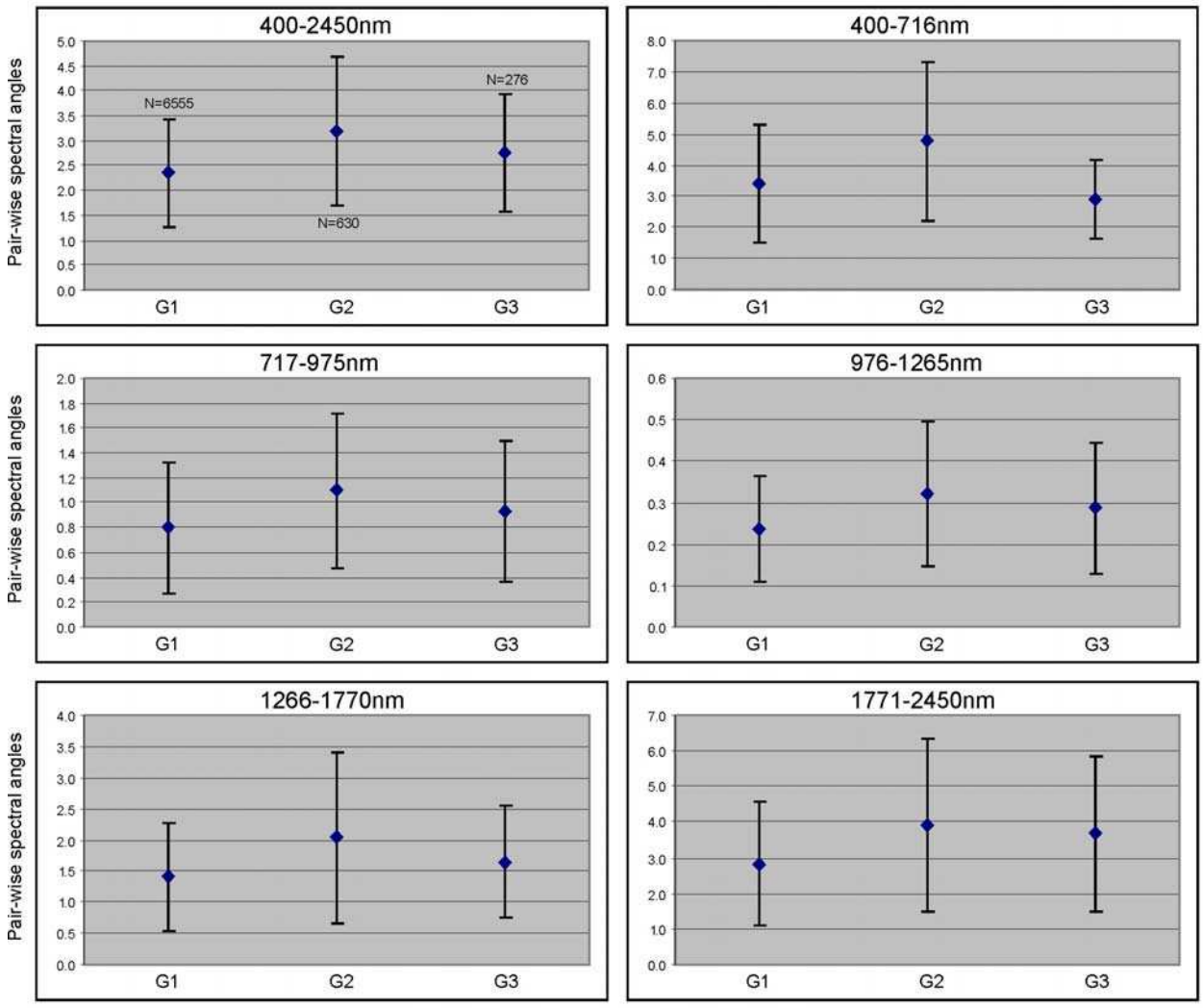


Figure 14. Mean pair-wise spectral angle (+/- 1 standard deviation) of leaf reflectance spectra for each wheat growth stage. Where pair-wise spectral angles are calculated for each pair of leaf spectra within a single growth stage. Number of pair-wise combinations computed for each growth stage is shown in the top left plot.

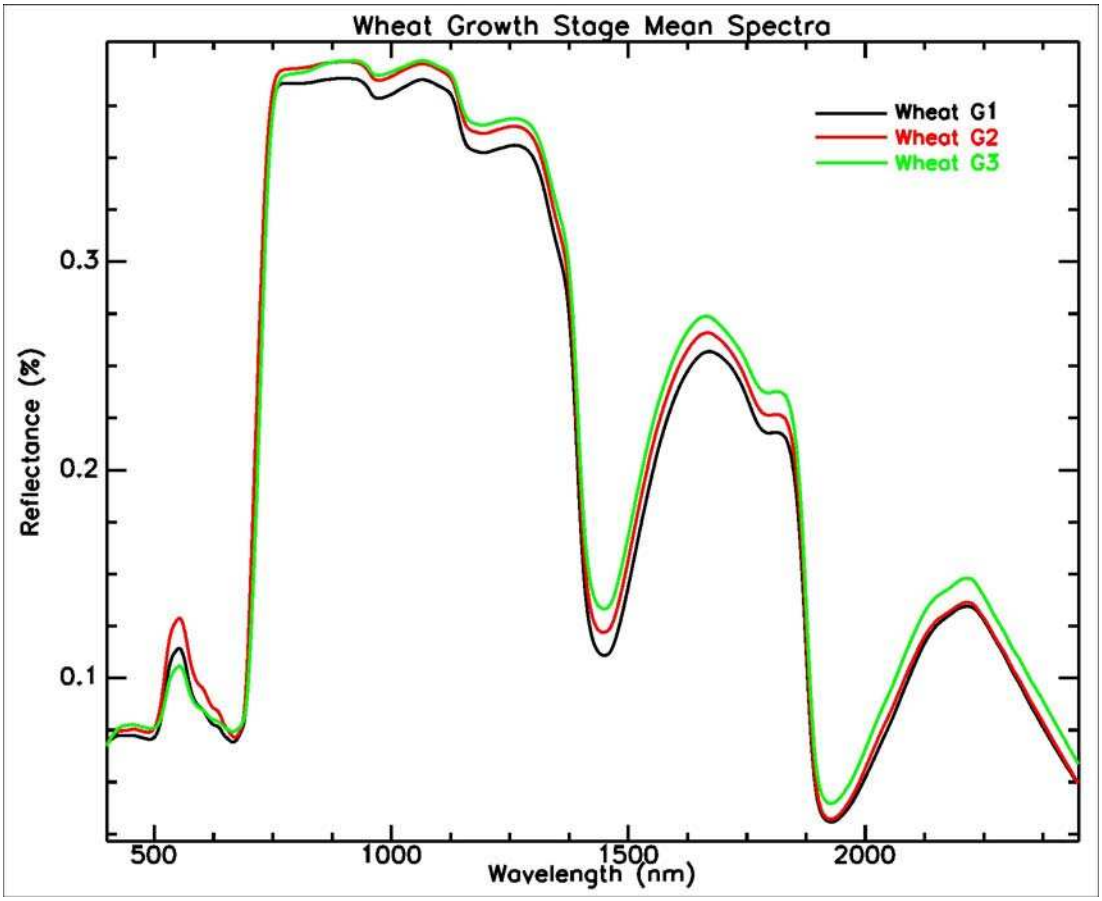


Figure 15. Mean reflectance spectra for each wheat growth stage.

Table 11. Spectral angle (degrees, 400-2450nm range) between mean spectra of each growth stage shown in Figure 15.

	G1	G2	G3
G1			
G2	**1.00**(1)		
G3	2.29	2.20	

(1) G1 and G2 most similar growth stages based on spectral angle.

7. RESULTS PHASE 2

7.1 Spectral significance of experiments

Measurements acquired using the ASD® FR, re-calibrated ASD® FR, and Hand Held ASD® were analyzed separately and results shown accordingly (if applicable). Table 12 shows the experiments that resulted in significant spectral changes (highlighted in bold) with respect to control plants. Note that canola and wheat experiments are split into those using the ASD® FR, re-calibrated ASD® FR, and Hand Held ASD®.

Table 12. List of experiments (in bold) that show significant spectral changes compared with control plants.

Poplar	$1H_2O24$[(1)]	**$1H_2O48$**	**$2H_2O24$**							
	1NaCl24	2NaCl24	2NaCl48	**3NaCl**	**4NaCl24**	**4NaCl48**				
	$1NH_3$24	$2NH_3$24	**$3NH_3$24**	**$3NH_3$48**						
Canola ASD	$1Cl_2$24	$1Cl_2$48	$2Cl_2$24	$2Cl_2$48	**$7Cl_2$24**	**$7Cl_2$48**				
	$1H_2O24$	**$1H_2O48$**	$2H_2O48$	$2H_2O72$	**$2H_2O96$**	**$2H_2O120$**				
	$1H_2S24$	$1H_2S48$	$2H_2S24$	$2H_2S48$						
	1HCN24	**1HCN48**								
	1NaCl24	**1NaCl48**	**2NaCl**	3NaCl24	3NaCl48					
	$1NH_3$24	$1NH_3$48	$2NH_3$24	$2NH_3$48	$3NH_3$24	**$3NH_3$48**	$4NH_3$24	**$4NH_3$168**	$5NH_3$24	**$5NH_3$48**
	$1SO_2$24	**$1SO_2$48**	**$2SO_2$24**	**$2SO_2$48**	**$3SO_2$24**	$3SO_2$48	**$8SO_2$24**	**$8SO_2$48**		
Canola HH ASD	$3Cl_2$24	**$3Cl_2$48**	**$4Cl_2$24**	**$4Cl_2$48**						
	$4SO_2$24	$5SO_2$24								
Canola re-calibrated data	$5Cl_2$24	$5Cl_2$48	**$6Cl_2$24**	**$6Cl_2$48**						
	$6SO_2$24	$6SO_2$48	**$7SO_2$24**	**$7SO_2$48**						
Wheat	$5Cl_2$24	**$5Cl_2$48**								
	$1H_2O48$	$1H_2O72$	**$1H_2O96$**	**$1H_2O120$**						
	1HCN24	**1HCN48**								
	1NaCl24	1NaCl48	2NaCl24	**2NaCl48**						
	$1NH_3$24	**$1NH_3$48**	$2NH_3$24	$2NH_3$48	$3NH_3$24	$3NH_3$48				
	$5SO_2$24	**$5SO_2$48**								
Wheat HH ASD	$1Cl_2$24	$1Cl_2$48	$2Cl_2$24	$2Cl_2$48						
	$4NH_3$24									
Wheat re-calibrated data	$3Cl_2$24	$3Cl_2$48	$4Cl_2$24	$4cCl_2$48						
	$3SO_2$24	$3SO_2$48	$4SO_2$24	**$4SO_2$48**						

(1) The number given at the end refers to the approximate measurement time (in hours) following treatment (e.g. 24 hours).

7.2 Endmember Analysis

7.2.1 Poplar

Results from the endmember analysis of poplar experiments showed that the applied treatments (NaCl and NH_3) and the dehydration stress (H_2O) had a significant effect on the reflectance spectra of leaves (Figure 16). Spectral changes were observed across the full spectral range used in this analysis. In the SWIR the predominant difference relative to a mature healthy leaf is an increase in reflectance. Absorption features, such as the broad one centered at ~ 2110 nm, become apparent because of dehydration (Carter, 1991) and are associated with cellulose and lignin. In addition, dehydration causes narrow absorption features that can be related to cellulose, oils, starches, and sugars (Curran, 1989) to become visible at ~1680, 1730, 2310 and 2350 nm. In the NIR the slope of the short wavelength end of the infrared plateau (~750 – 1100 nm) decreases. The slope change varies but is significant in all experiments. In the visible the key change is a deepening of the chlorophyll trough owing to increased reflectance between 550 and 650 nm, which can be attributed to chlorophyll (A, B) loss (chlorosis). As such, a number of narrow absorption features become visible (e.g. ~540 and 615 nm) where these features are related to other pigments such as ß-carotene, pycoerythrin and phycocyanin. Figure 17 shows some examples of leaves under high stress from H_2O, NaCl, and NH_3.

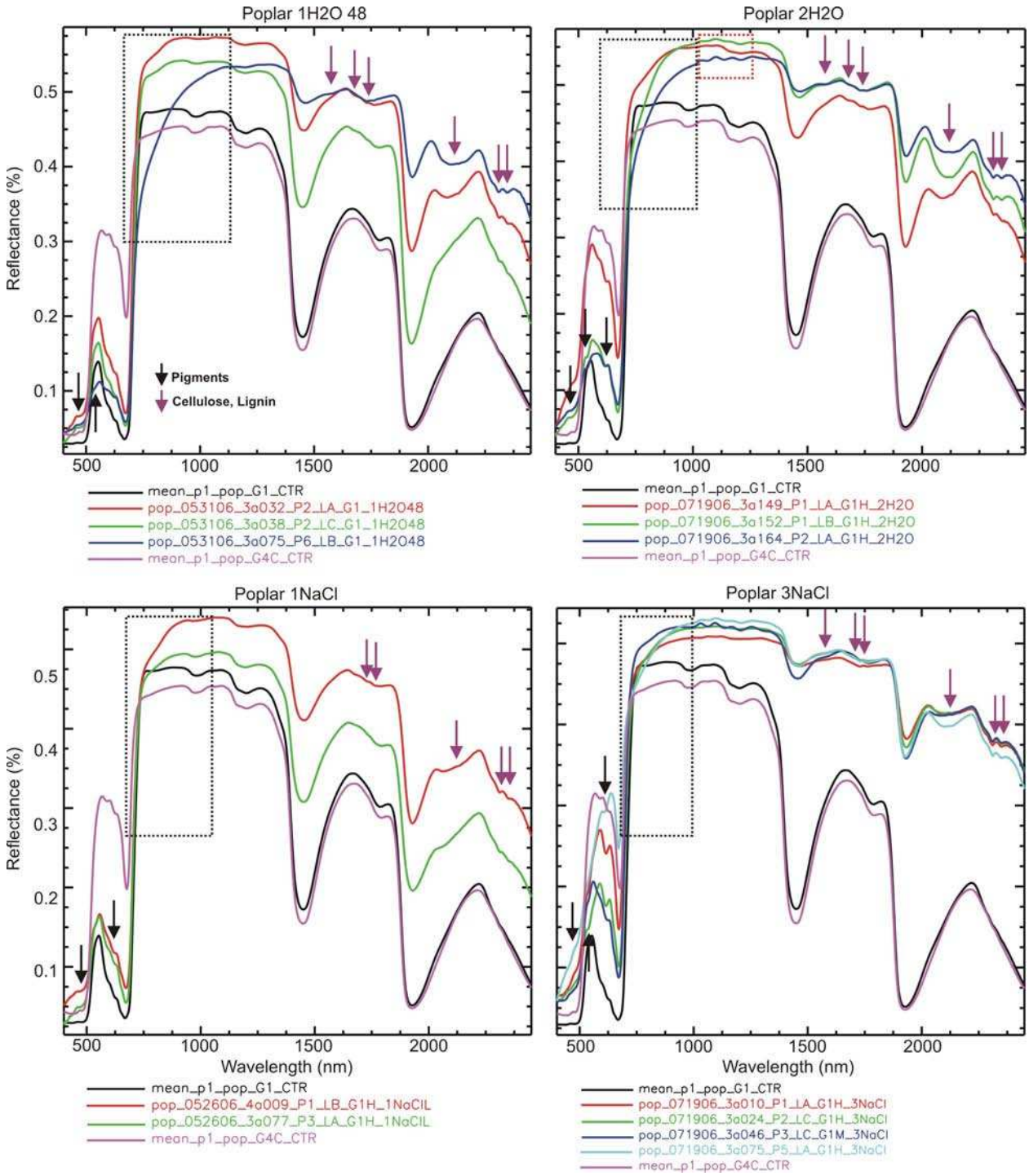


Figure 16. Endmember spectra for poplar experiments H_2O, NaCl and NH_3. Mean spectra of control G1 (black line, mature healthy) and G4C (pink line, high senescence) are included for reference. Arrows denote key absorption features observed in endmember spectra compared with G1. Black dotted box denotes smoothing of red edge. Red dotted box shows example of the 976 to 1265 nm range for the instrument problem noted in section 4.1.1.

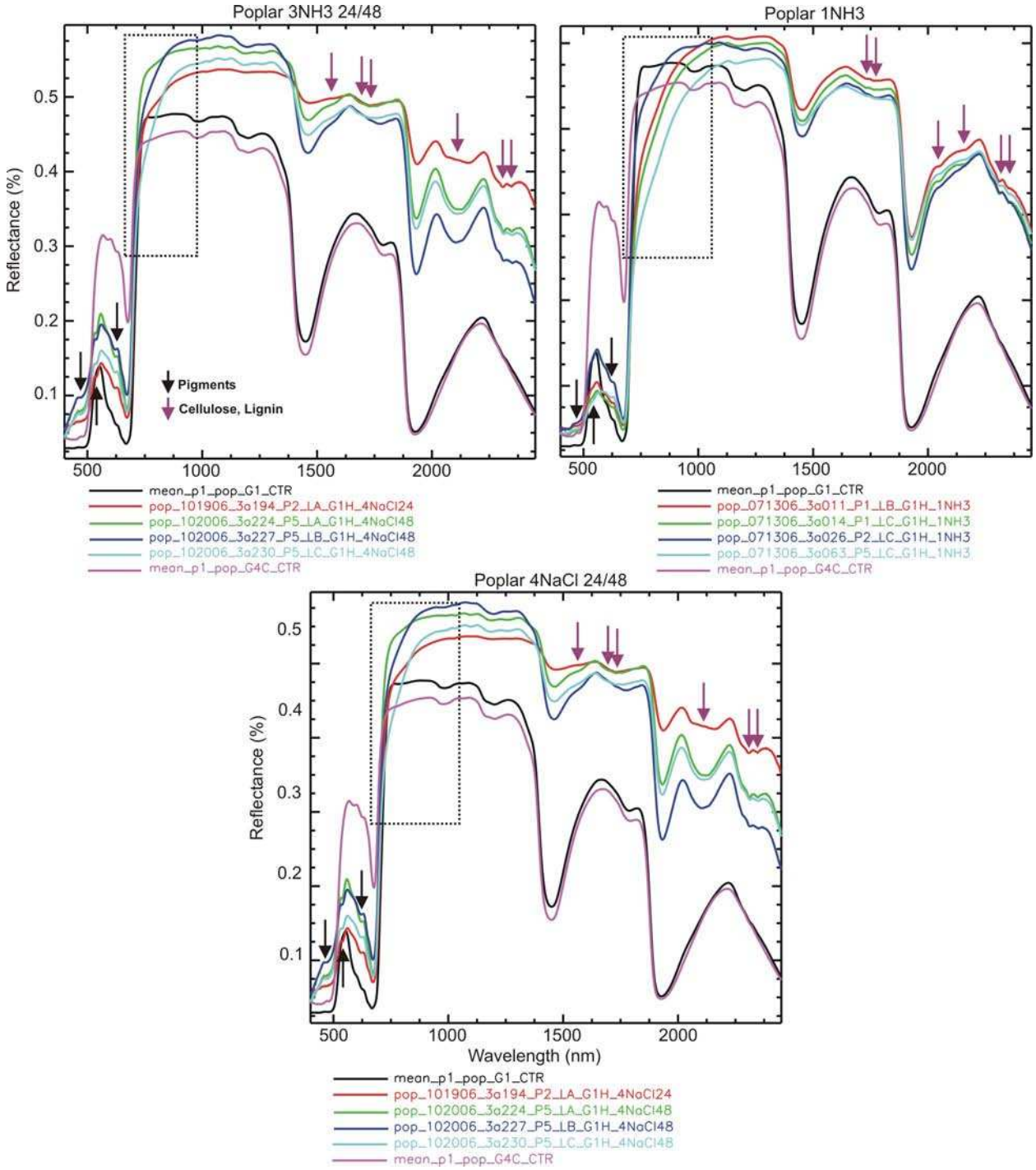


Figure 16 cont' Endmember spectra for poplar experiments H_2O, NaCl and NH_3. Mean spectra of control G1 (black line, mature healthy) and G4C (pink line, high senescence) are included for reference. Arrows denote key absorption features observed in endmember spectra compared with G1. Black dotted box denotes smoothing of red edge.

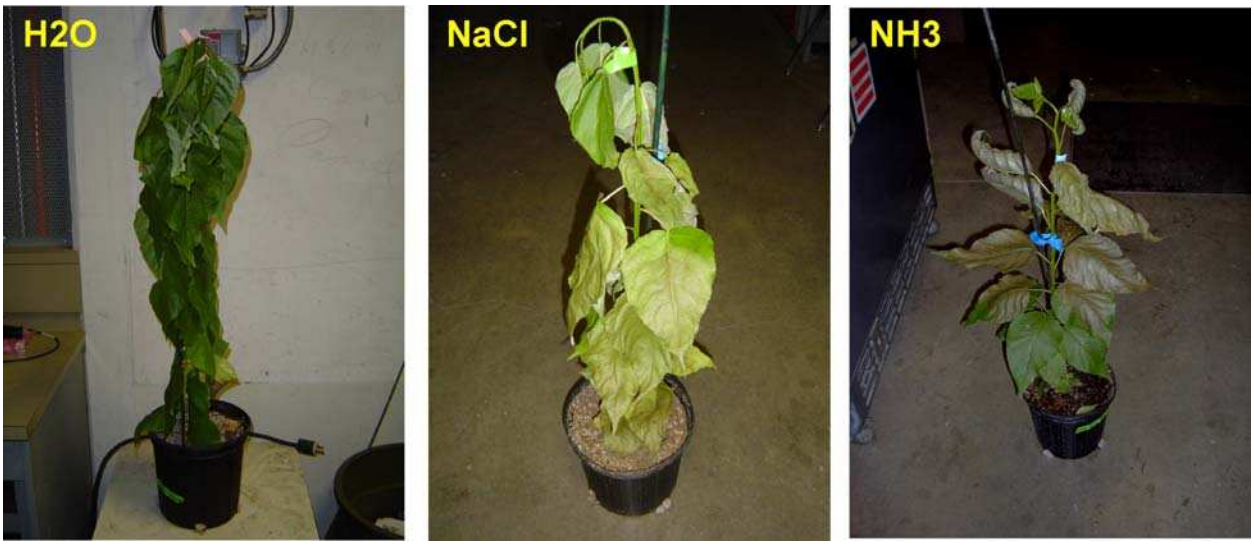


Figure 17. Examples of visible changes in poplar leaves under high stress from H_2O, NaCl, and NH_3.

7.2.2 Canola

Results from the endmember analysis of canola experiments showed that not all of the applied treatments (NaCl, H_2S, HCN, NH_3, Cl_2, and SO_2) and dehydration stress (H_2O) had a significant effect on leaf reflectance spectra (Figure 18). Treatments that show significant changes across the full spectral range were NH_3, Cl_2, and SO_2, with significant changes only occurring over a small portion of the spectral range observed for NaCl, H_2S, HCN, and dehydration. For NH_3, Cl_2, and SO_2 treatments similar spectral features in the visible (chlorophyll and pigments) and in the SWIR (lignin, cellulose, oils, starches, and sugars) as those described for poplar were observed, but as with poplar, the depth of an absorption feature, the increase in reflectance, or the slope change of the red edge varied. For the NaCl, H_2S, HCN, and dehydration experiments the key changes are an increased slope between the green peak and chlorophyll trough, and, an overall minor increase in reflectance in the SWIR. The latter is not always easily visible as the absolute reflectance between measurements may vary owing to subtle illumination variability (refer to section 4.1.1). Figure 19 shows some examples of leaves under high stress from NH_3, Cl_2, and SO_2.

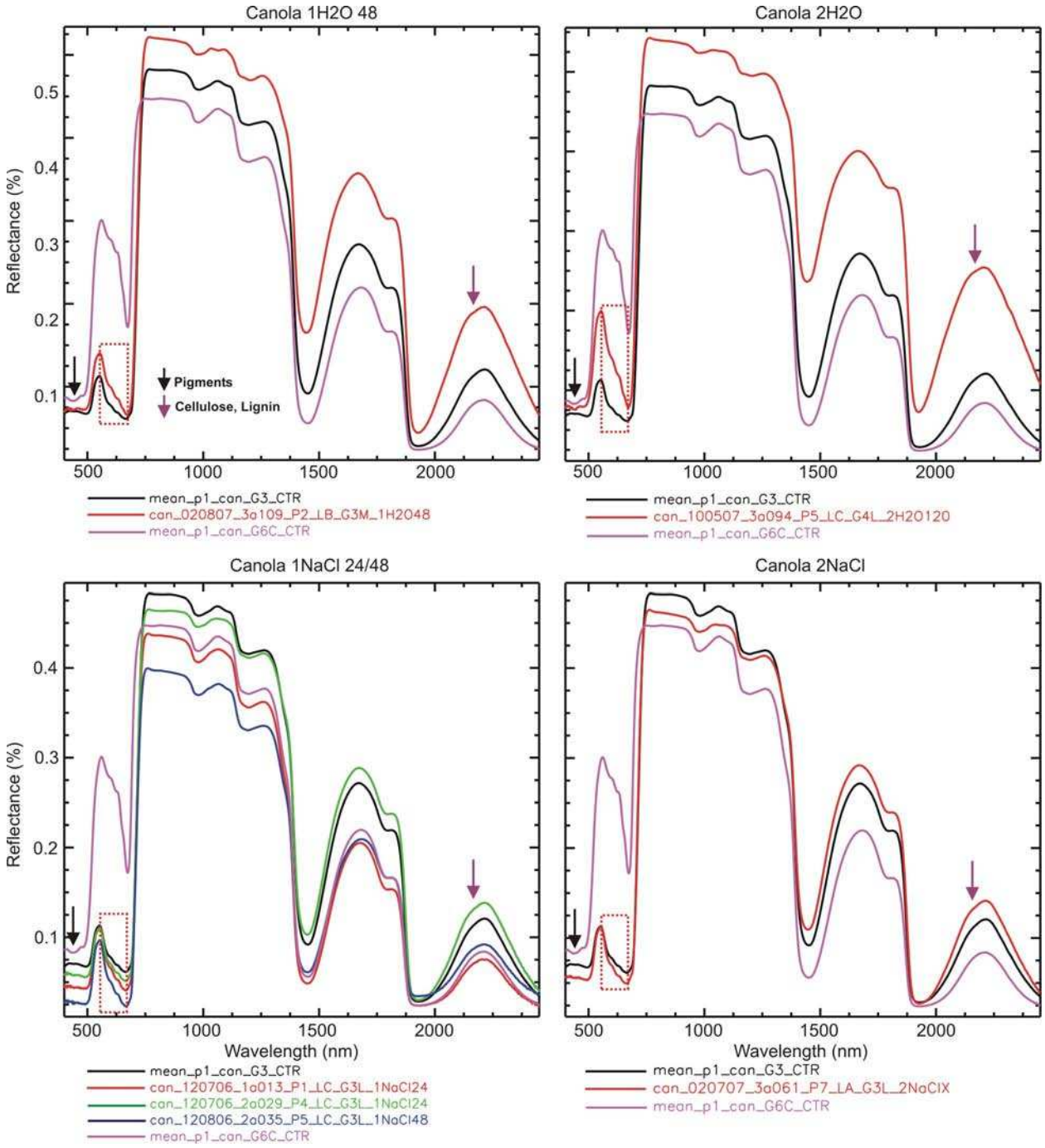


Figure 18. Endmember spectra for canola experiments H_2O, NaCl, HCN, and NH_3. Mean spectra of control G3 (black line, mature healthy) and G6C (pink line, high senescence) are included for reference. Arrows denote key absorption features observed in endmember spectra compared with G3. Red dotted box denotes an increased ratio between the green peak and chlorophyll trough.

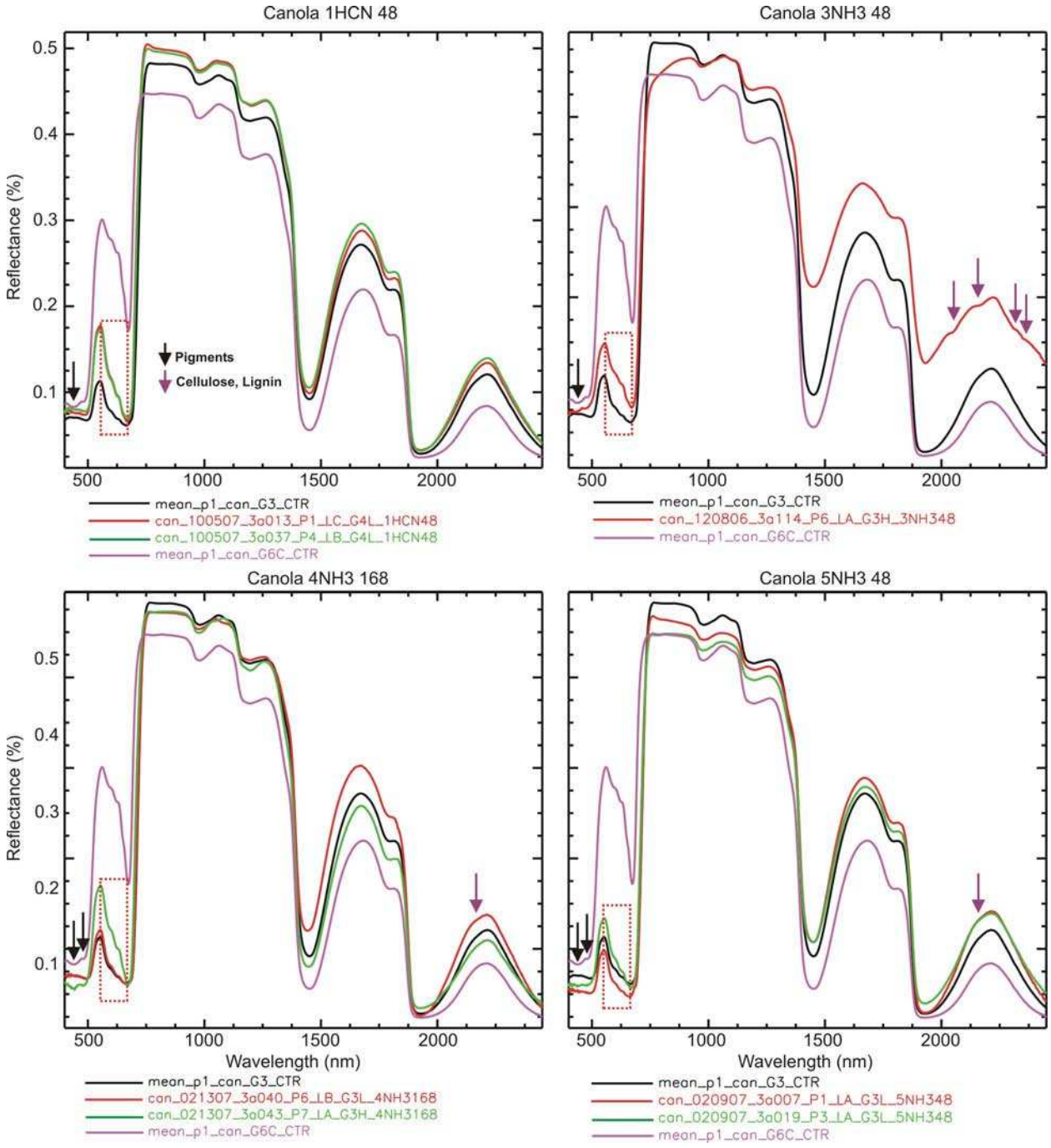


Figure 18 cont' Endmember spectra for canola experiments NH_3, Cl_2 and SO_2. Mean spectra of control G3 (black line, mature healthy) and G6C (pink line, high senescence) are included for reference. Arrows denote key absorption features observed in endmember spectra compared with G3. Black dotted box denotes smoothing of red edge. Red dotted box denotes an increased ratio between the green peak and chlorophyll trough.

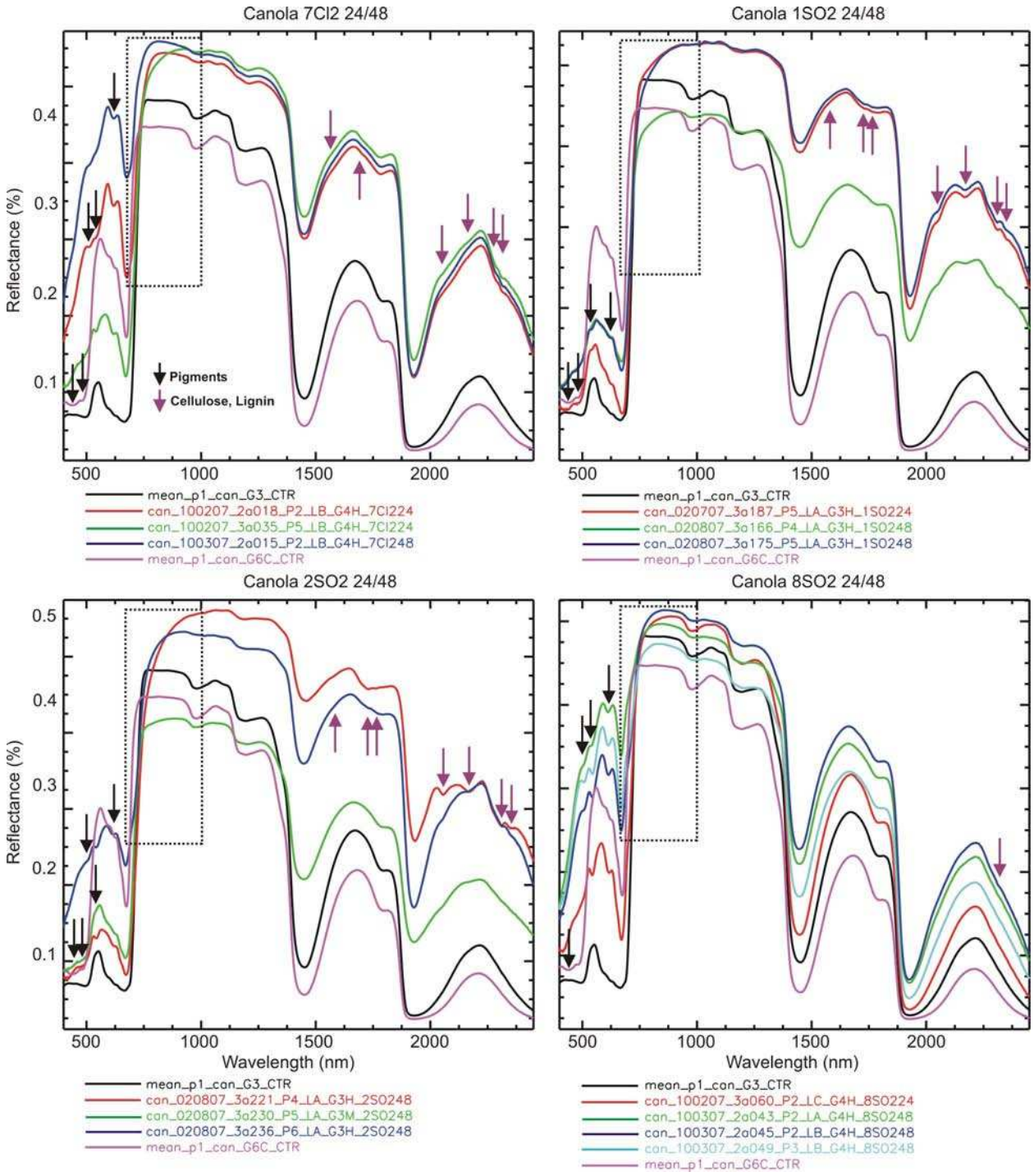


Figure 18 cont' Endmember spectra for canola experiments NH_3, Cl_2 and SO_2. Mean spectra of control G3 (black line, mature healthy) and G6C (pink line, high senescence) are included for reference. Arrows denote key absorption features observed in endmember spectra compared with G3. Black dotted box denotes smoothing of red edge. Red dotted box denotes an increased ratio between the green peak and chlorophyll trough.

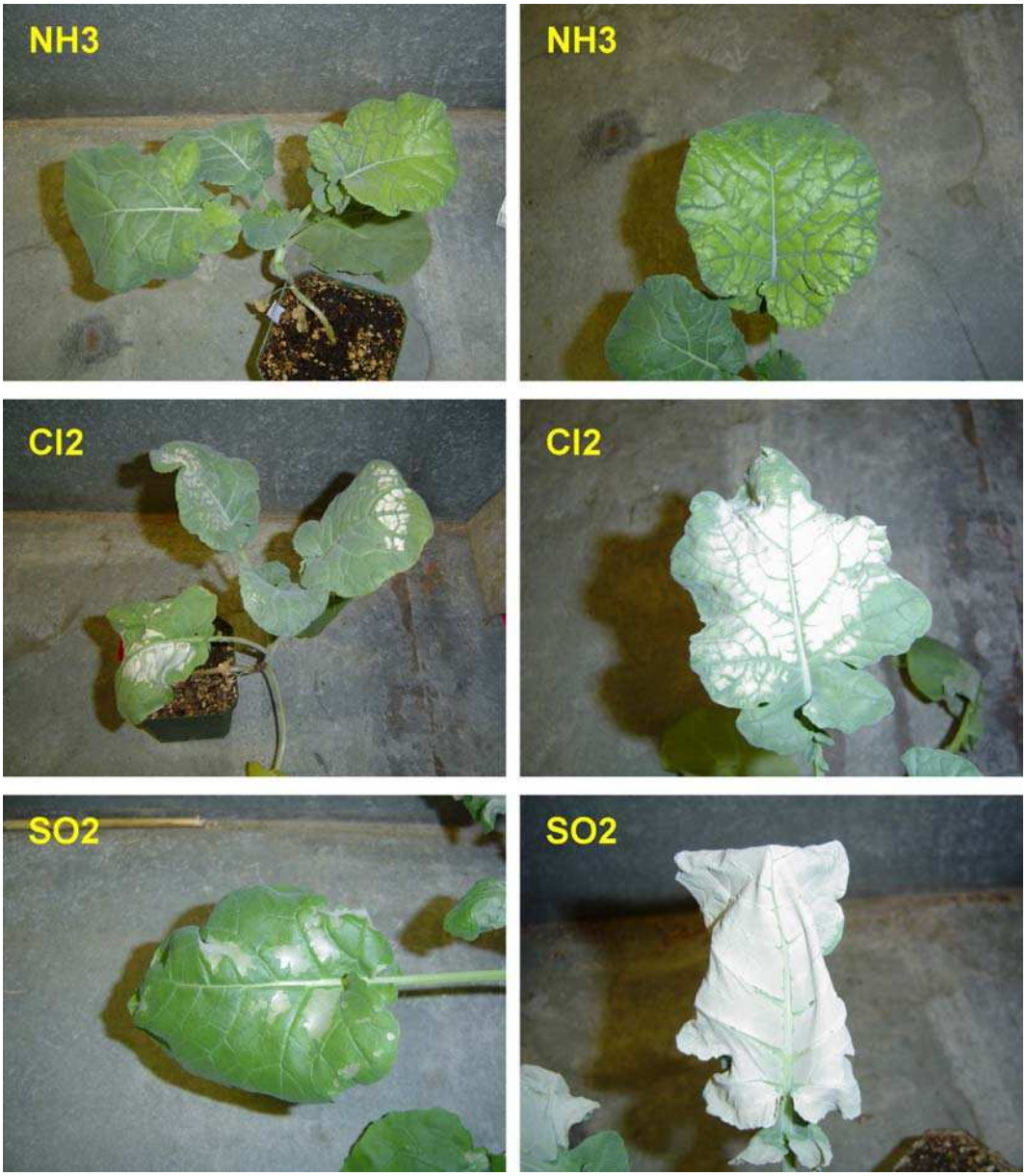


Figure 19. Examples of visible changes in canola leaves under medium and high stress (left and right columns) from NH_3, Cl_2, and SO_2.

7.2.3 Wheat

Wheat response to the various treatments and dehydration stress was minimal compared to poplar and canola. Results from the endmember analysis showed that the applied treatments (NaCl, HCN, NH_3, Cl_2, and SO_2) and dehydration stress (H_2O) do not all have a significant effect on the plants (Figure 20). Treatments that showed significant changes across the full spectral range were restricted to HCN, SO_2 and H_2O with significant changes only occurring over a small portion of the spectral range observed for NaCl, NH_3 and Cl_2. For treatments HCN and SO_2, and dehydration, similar spectral features as those described for poplar and canola were observed in the SWIR (lignin, cellulose, oils, starches, and sugars). Notable spectral changes in the visible (chlorophyll and pigments) and NIR (red edge slope) were restricted to the SO_2 treatment. For the NaCl, Cl_2 and NH_3 treatments minor spectral changes included an increased slope between the green peak and chlorophyll trough, and, a minor increase in reflectance in the SWIR. Figure 21 shows some examples of leaves under high stress from HCN, SO_2 and dehydration.

7.2.4 Implications of endmember analysis

The spectral features observed in Figures 16,18 and 20 show that the TIC treatments and environmental stresses (NaCl and dehydration) result in similar spectral responses. Many of the features become visible primarily because of chlorosis (visible) and dehydration (SWIR). This is not unexpected as plant physiological responses to stress are similar regardless of the cause of stress (Chaplin, 1991). However, depending on the level of stress experienced by a given plant, the depth of a given absorption feature, the increase in reflectance, or the slope change of the red edge varies. These differences reflect how a plant, or individual leaf, responds over time to the exposure of a given treatment. Thus, in the next section we observed how the spectral response of treated leaves varied from relatively healthy to extreme stress-induced damage.

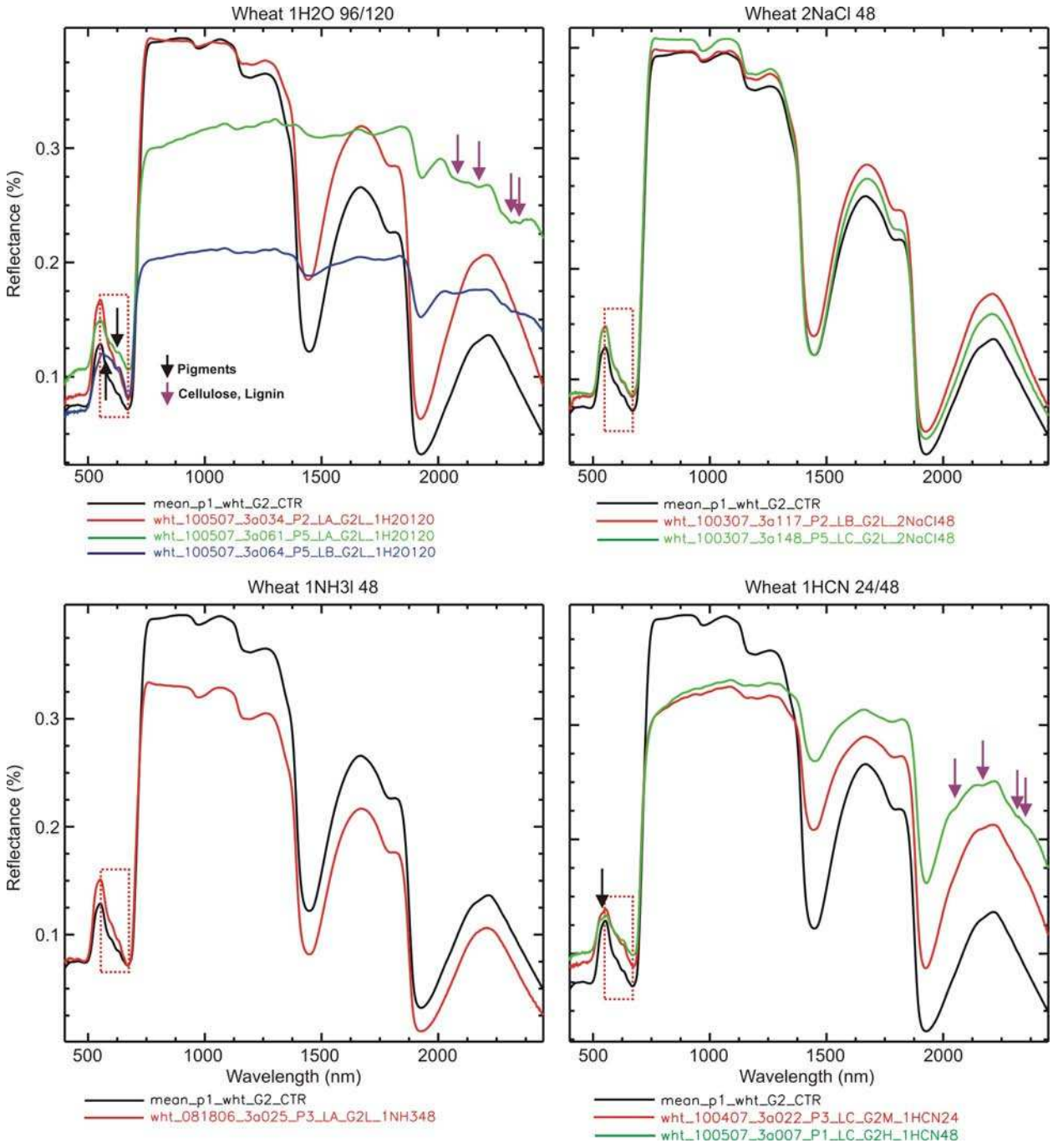


Figure 20. Endmember spectra for wheat experiments H_2O, NaCl HCN, NH_3, Cl_2 and SO_2. Mean spectra of control G2 (black line, mature healthy) is included for reference. Arrows denote key absorption features observed in endmember spectra compared with G3. Black dotted box denotes smoothing of red edge. Red dotted box denotes an increased ratio between the green peak and chlorophyll trough.

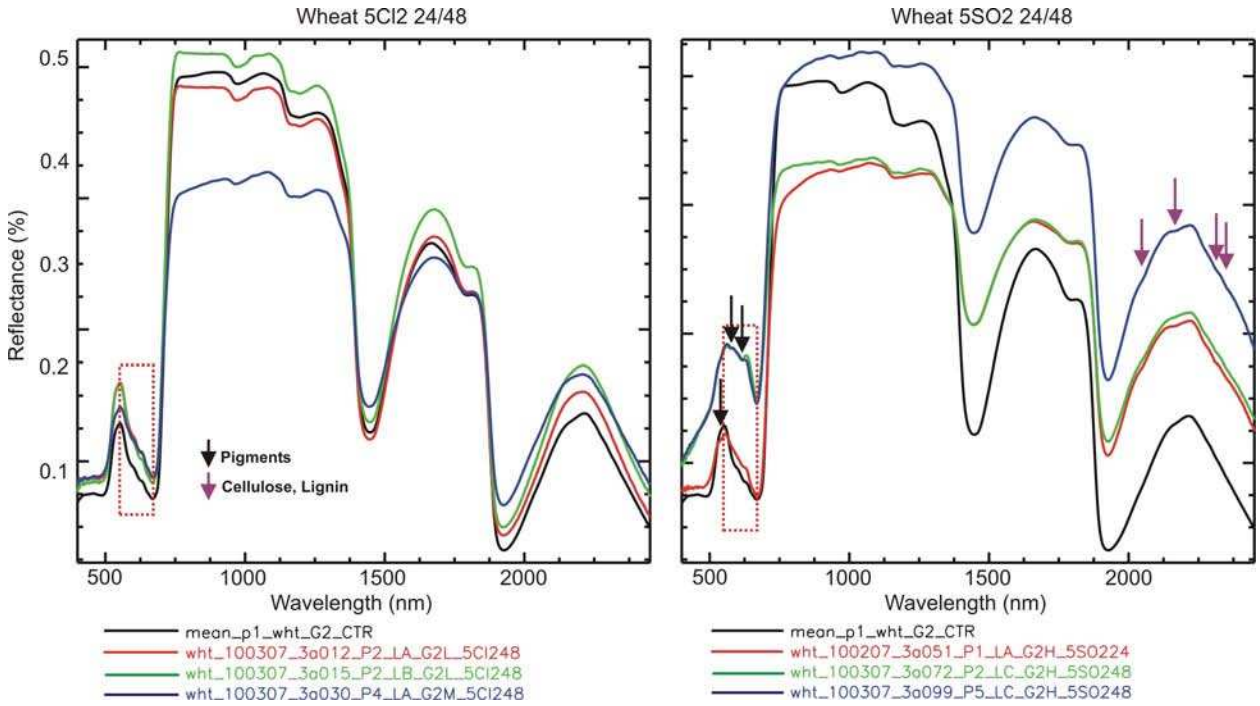


Figure 20 cont' Endmember spectra for wheat experiments H_2O, NaCl HCN, NH_3, Cl_2 and SO_2. Mean spectra of control G2 (black line, mature healthy) is included for reference. Arrows denote key absorption features observed in endmember spectra compared with G3. Black dotted box denotes smoothing of red edge. Red dotted box denotes an increased ratio between the green peak and chlorophyll trough.

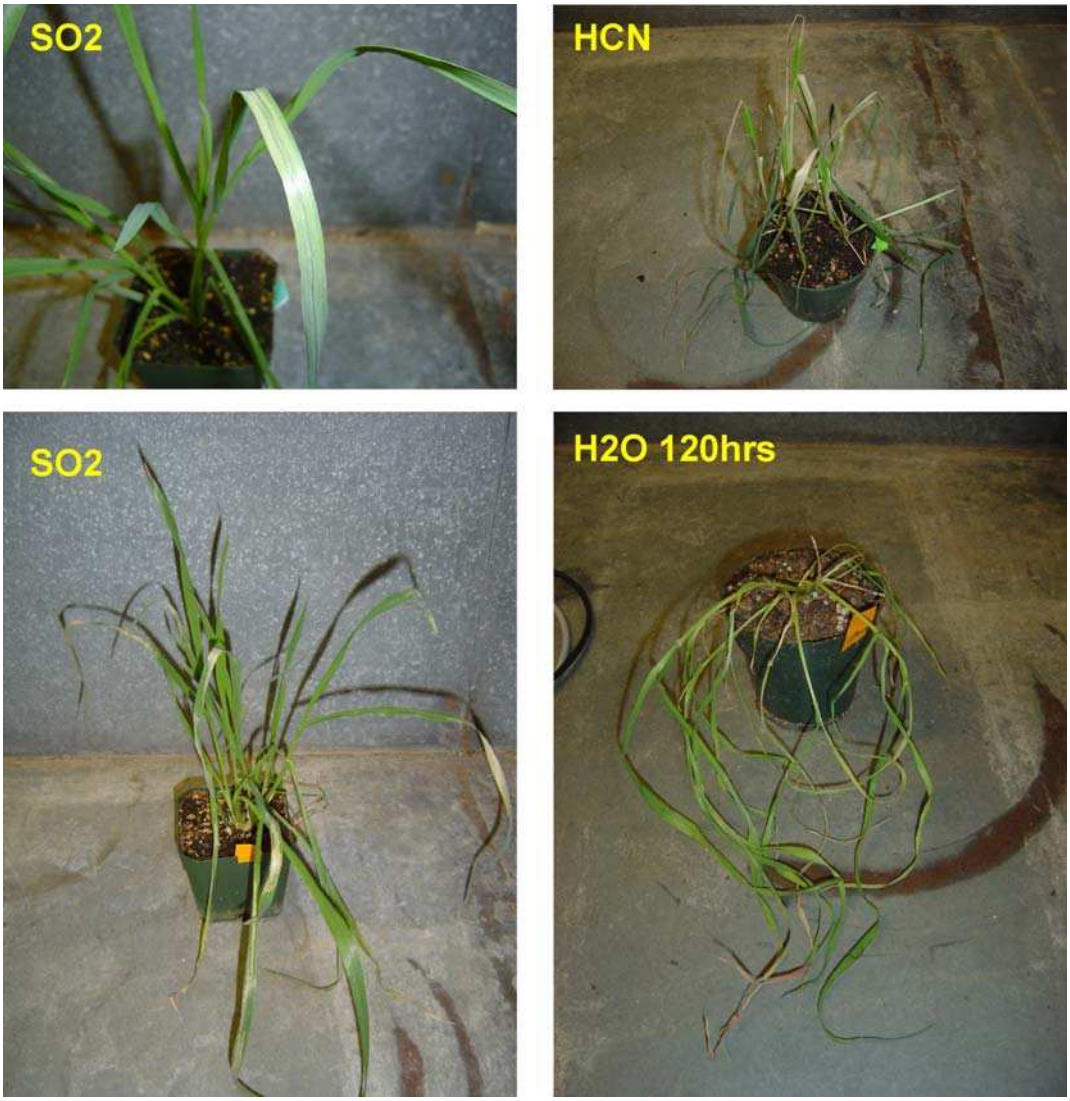


Figure 21. Examples of visible changes in wheat leaves under high stress from SO_2, HCN, and dehydration at 120 hrs.

7.3 Vegetation Indices

The previous section showed that the various treatments do not result in a diagnostic absorption feature (or features) that is specific to a given treatment. The features that are observed are inherent spectral features of the plants that are no longer masked by the dominant spectral features, such as chlorophyll in the visible and water in the SWIR. However, the relative rates of chlorophyll and water loss may vary between TICs, resulting in the variability of the depth of a given absorption feature, the increase in reflectance, or the slope change of the red edge. In this section we made use of vegetation indices (see Table 5) that focus on bands related to chlorophyll, pigments and water content of leaves in order to analyze the trend taken by a group of plants from healthy mature to highly stressed. The objective was to determine if specific TIC treatments resulted in diagnostic trends, with respect to other treatments and with respect to natural stress (e.g. NaCl, dehydration and senescence).

For each type of treatment all experiments that showed significant spectral changes (see section 5.2.1) were combined into a single spectral library for subsequent analysis using vegetation indices. This was done to capture a well-defined trend from healthy to highly stressed leaves. The following results focus on measurements acquired using the ASD® FR. The associated control plants for each treatment were also combined into a single library. Senesced leaves for poplar and canola from Phase 1 were included in this analysis. Vegetation indices were calculated for each compiled library and were plotted against each other to highlight trends that vary between environmental stresses and treatments, and/or between treatments. Only environmental stresses and treatments that resulted in notable trends lines are shown in this section.

7.3.1. Poplar vegetation indices

Figure 22 shows a series of vegetation indices scatter plots for poplar treatments NaCl and NH_3, dehydration (H_2O), senescence, and controls plants. The results clearly demonstrated that trend lines related to NH_3 and senescence were distinguishable from NaCl. H_2O followed a trend that is similar to NaCl in most plots, however, it also showed a fair degree of variability resulting in an overlap with NH_3 and senescence.

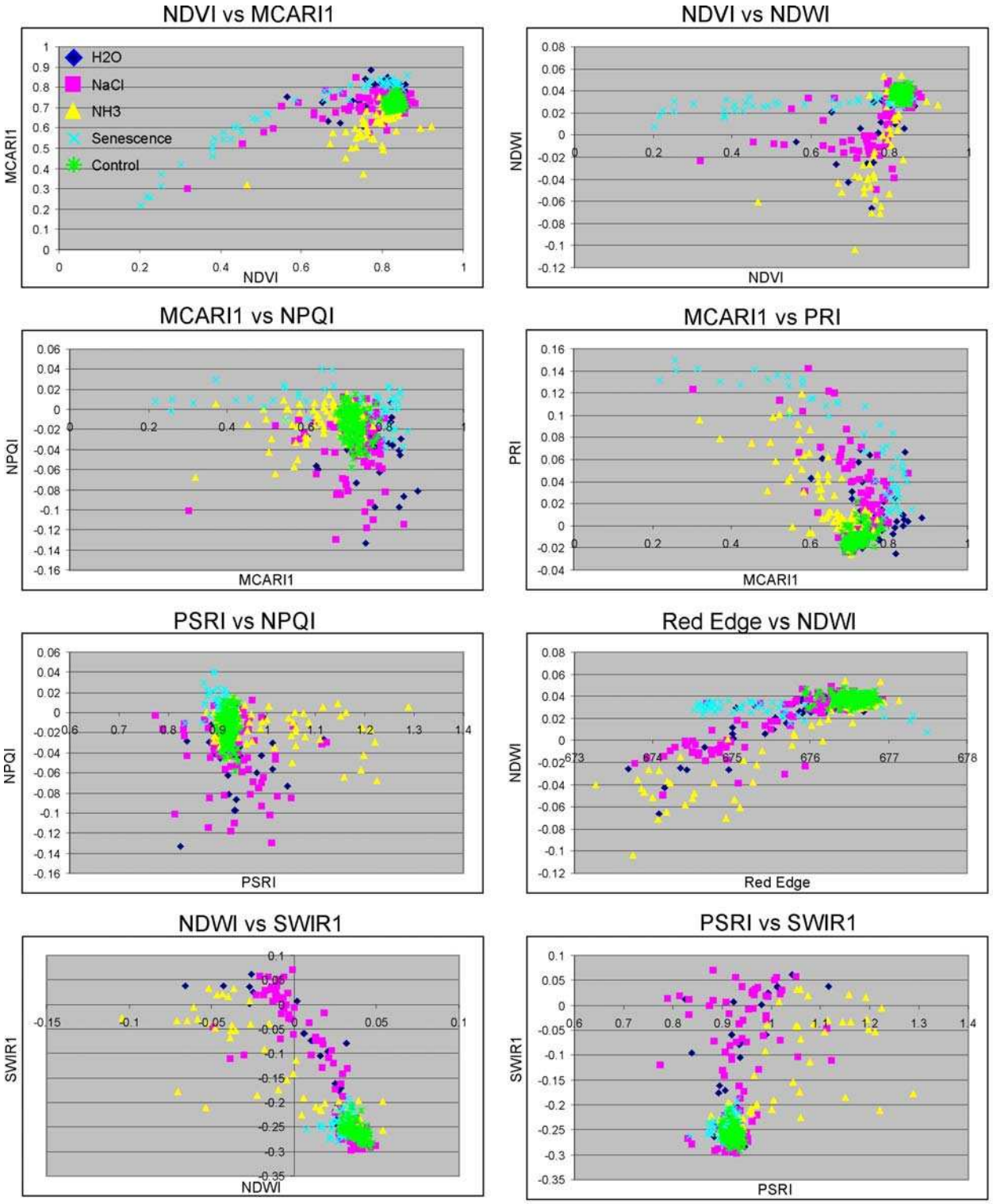


Figure 22. Vegetation indices for poplar treatments NaCl and NH_3, dehydration (H_2O), senescence, and controls plants. Refer to Table 5 for details on each indice.

7.3.2. Canola vegetation indices

Figure 23 shows vegetation indices scatter plots for canola treatments Cl_2 and SO_2, dehydration (H_2O), senescence, and controls plants. The various plots showed that Cl_2 and SO_2 treatments could be distinguished from dehydration and senesced plants. In the plots NDVI vs Red Edge, MCARI1 vs Red Edge, and PSRI vs NPQI, Cl_2 and SO_2 showed slightly different trends and were distinguishable. However, discrimination between Cl_2 and SO_2 was not possible in the other plots, which showed a significant degree of overlap between the two treatments. In the plot PSRI vs SWIR1 it appeared that SO_2 was split into two different trends. A more in depth analysis of this plot and the plot NDVI vs NDWI supported this observation, thus we examined the results for individual SO_2 experiments in Figure 24. The dosage applied to the three experiments were ~50 ppm (exp 1), ~100ppm (exp 2), and 60-120 ppm (exp 3), respectively. Experiment 1 and 2 show a similar trend, but differ in dosage. Experiment 8 shows a different trend from both 1 and 2, but had variable dosage similar to 1 and 2 combined. Thus, dosage does not appear to have been the controlling factor on the trends observed between the different SO_2 experiments. Closer examination of the NDVI vs NDWI plot showed that the key difference between the SO_2 experiments was water content (as expressed by NDWI). Thus, we could speculate that initial water content may have been the controlling factor in the trends observed in these experiments rather than the treatment type.

7.3.3. Wheat vegetation indices

The results from the vegetation indices scatter plots for wheat (Figure 25) did not show trends that were as well-defined as for poplar and canola plants. This was not unexpected as the wheat plants were consistently less affected by application of TICs used in this project. There was considerable scatter for the wheat, however, general trends that discriminate SO_2, HCN, and dehydration were visible in the plots NDVI vs NDWI, PRI vs NDWI, PSRI vs NDWI, NDWI vs SWIR1, and PRI vs SWIR1. In each of these cases dehydration appeared to be an important discriminating feature as expressed by NDWI and SWIR1. A slightly different trend was also visible between dehydration and SO_2 and HCN in the PRI and SWIR1 plot. Cl_2, which did not show as extreme a response as with HCN, SO_2, and dehydration, still showed a few points that appeared to follow the trend of SO_2.

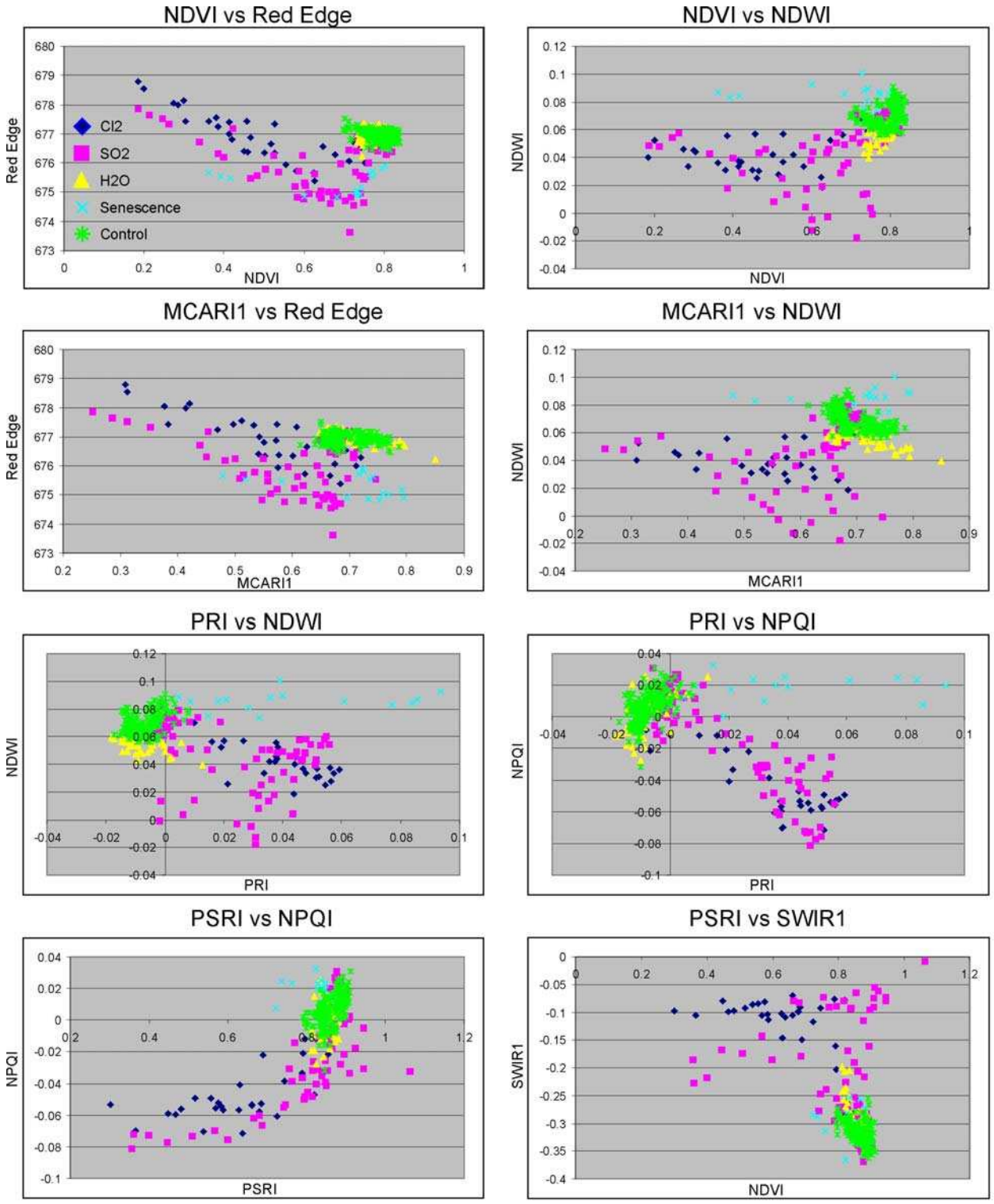


Figure 23. Vegetation indices for canola treatments Cl_2 and SO_2, dehydration (H_2O), senescence, and controls plants. Refer to Table 5 for details on each indice.

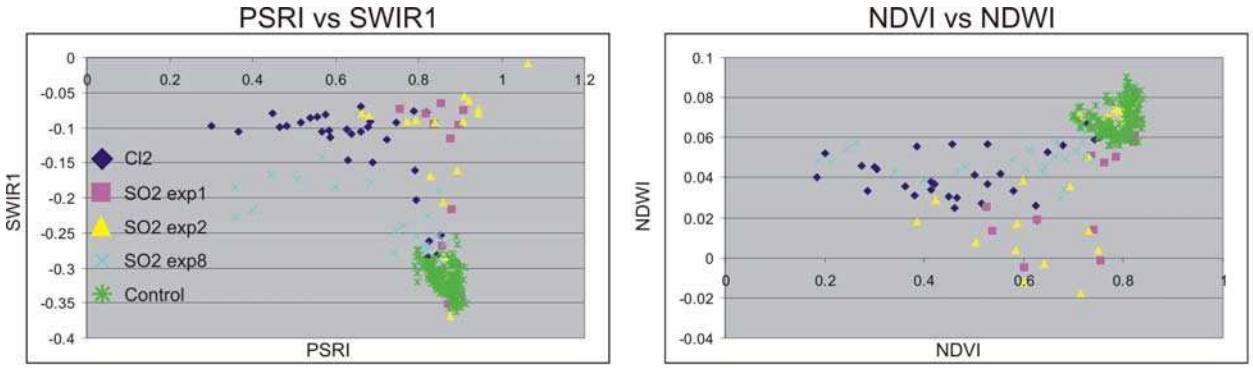


Figure 24. Vegetation indices PSRI vs SWIR1 and NDVI vs NDWI for canola treatments Cl_2 and SO_2, and controls plants. SO_2 has been separated into individual experiments. Refer to Table 5 for details on each indice.

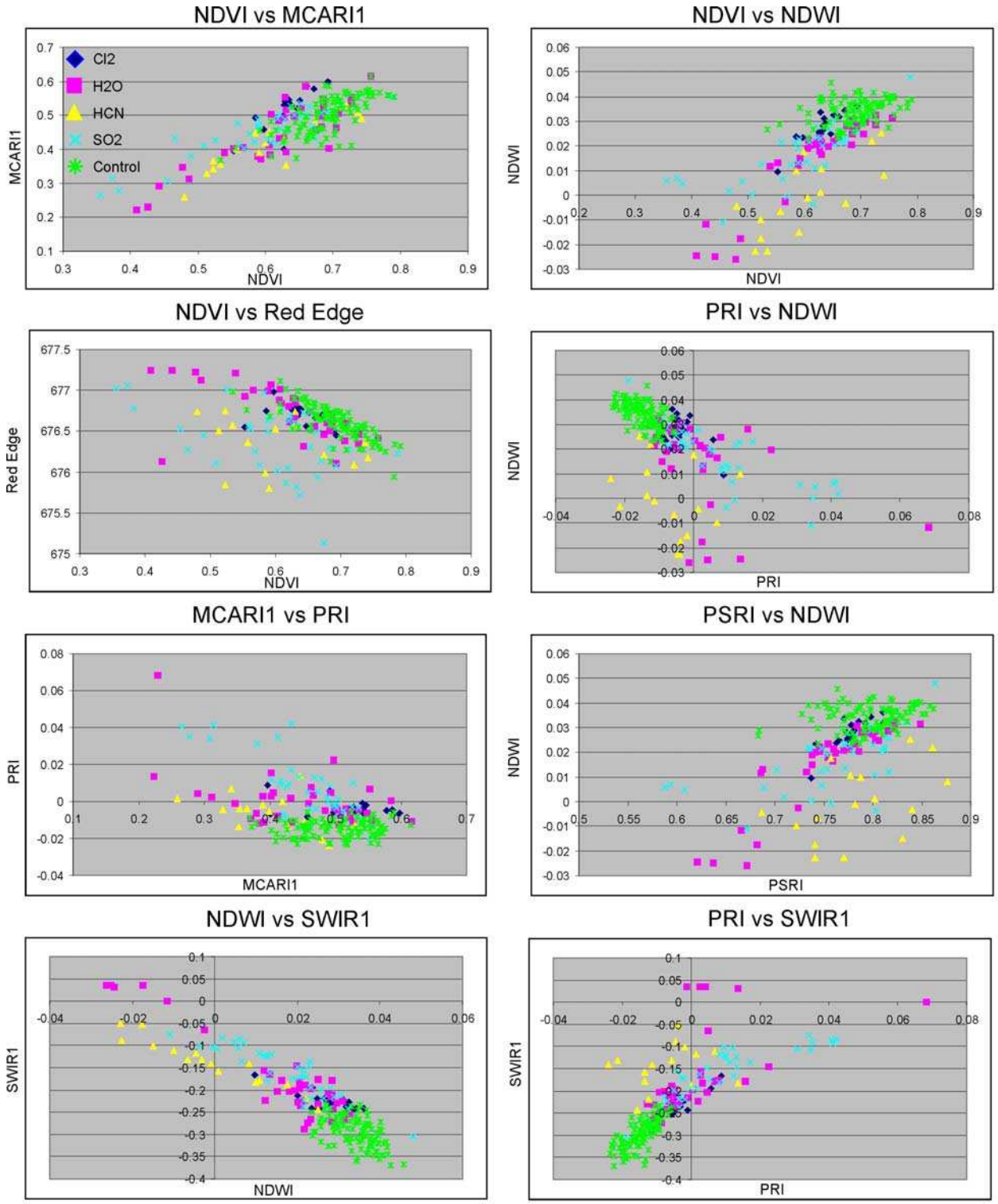


Figure 25. Vegetation indices for wheat treatments Cl_2, HCN, SO_2, dehydration (H_2O), and controls plants. Refer to Table 5 for details on each indice.

7.3.4. Indices across species

Vegetation indices, with respect to each species, show that it is possible to distinguish between environmental stresses and TICs, and in some cases between TICs. However, the next question is whether the trends observed in the previous Figures show consistency across species. Figure 26 shows a selection of vegetation indices across all species for treatments NaCl, NH_3, SO_2, Cl_2, HCN, and, dehydration (H_2O), senescence and controls plants. In Figure 26 plot A (NDVI vs NDWI) it is difficult to determine any trends that are consistent across species. The key reason is that the control samples for each species plot as distinct clusters. Thus, trends from healthy to highly stressed leaves for each species have different starting points, which causes overlap with other data trends and makes discrimination of treatment and stresses difficult. In the remaining plots the control samples for the three species overlap to varying degrees, such that the trends observed begin close to the same starting point. In these plots it is evident that the environmental and TIC treatments show distinctive trends that are not a function of the species. This is most evident in plots E-F, where a combination of indices distinguishes NH_3, SO_2, and Cl_2 (from everything else) but not HCN. In the case of NaCl and H_2O the two tend to plot together in each example. This is not unexpected as NaCl induces ionic stress and an osmotic stress response that is common to drought. It is important to note that not all species included all TIC treatments, and as such, it is possible that differences could occur across species for a given TIC. However, based on the results accumulated in this study and shown in Figure 26 it appears that an appropriate set of vegetation indices (where control samples overlap) allows discrimination between environmental stresses and TICs, and in some cases between TICs.

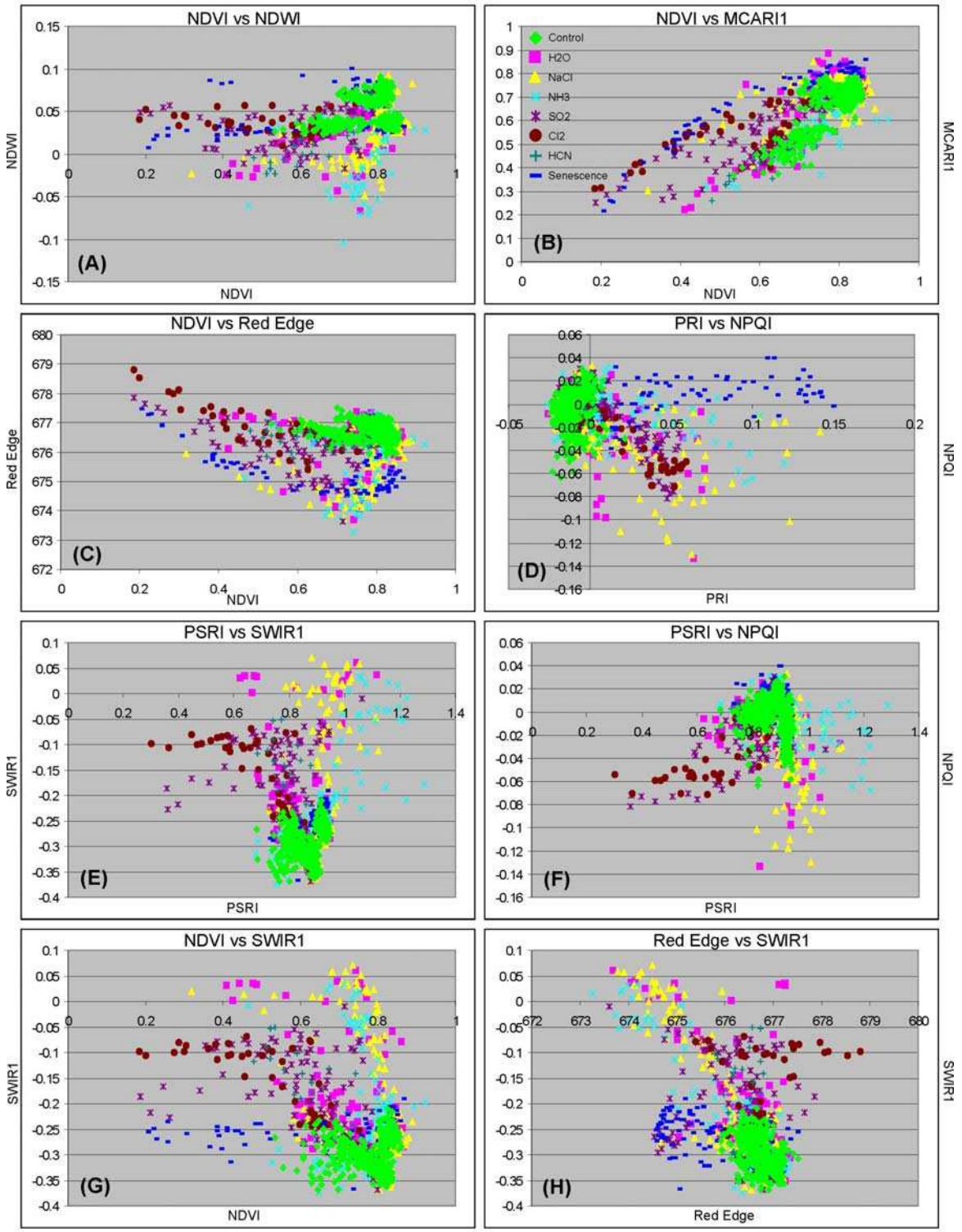


Figure 26. Vegetation indices across all species for treatments NaCl, NH_3, SO_2, Cl_2, HCN, and, dehydration (H_2O), senescence and controls plants. Refer to Table 5 for details on each indices.

8. DISCUSSION

8.1 Summary

This research team examined the spectral response of individual leaves of three common Canadian plant species: poplar (*Populus deltoides, Populus trichocarpa*); wheat (*Triticum aestivum)*; and canola (*Brassica napus*). These were subjected to fumigation with gaseous phase TICs. The two objectives of this study were to determine if: 1) vegetation subjected to TICs could be distinguished from background vegetation during varying growth stages (new growth to senescence) and environmental stresses; and, 2) different TICs could be distinguished based on the vegetation spectral response. Treatments were designed to allow quantification of the variation in spectra that might be expected due to environmental, developmental, and stochastic effects on the physiological state of individual plants within each species. All plants were grown in controlled environment chambers at the University of Alberta, using standardized conditions.

The study was broken into two phases: 1) to capture the spectral variability of the various growth stages observed in each of the three plant types; and 2) subjecting the plants to toxic industrial chemical (TIC) and environmental stresses. In Phase 1 we determined which growth stage of each species would be subjected to Phase 2 treatments based on spectral variability between growth stages and spectral similarity. In Phase 2 plant species were exposed to the following five TICs: ammonia (NH_3), sulphur dioxide (SO_2), hydrogen sulphide (H_2S), chlorine (Cl_2), and hydrogen cyanide (HCN). The experimental data were analyzed to determine if the various treatments resulted in specific spectral features related to TICs. The results showed that both environmental and TICs induced similar spectral features inherent to plants that can be related primarily to chlorophyll and water loss. These include pigments in the visible and cellulose, lignin, lipids starches, and sugars in the SWIR. Although no specific spectral features could be tied to individual TICs an analysis of the data using vegetation indices, which focus on key spectral bands associated with chlorophyll, pigments and water content, showed that the TICs and environmental stresses result in diagnostic trends from healthy mature to highly stressed leaves. In addition, further analysis showed that combinations of specific indices could be used to distinguish NH_3, SO_2, Cl_2 (from everything else) from each other consistently across all three species.

8.2 Laboratory experimental issues and relevance for airborne detection

This study was conducted at the leaf level to ensure good experimental control. We noted that variation in the spectral properties of individuals of some species could be detected within treatment groups and between replicate treatments. Although the sources of this variation is unknown, we speculate that a major component can be attributed to slight differences in water status of individual plants resulting from the sum of minor variations in ambient humidity, respiration rate, stomatal opening, soil evaporation, time since watering, light intensity (which decreases as fluorescent tubes age), and minor stochastic events. Because all plants in this study were grown under nominally identical conditions within controlled environment chambers, we expect that in the field, differences between individual plants could be magnified. To determine whether the net effect of this variation would be significant at the canopy level requires further experimentation. Detection of chemical effects on vegetation from an airborne platform will likely also be impacted by natural variability in water content and other factors over a given area. Thus, the results from this analysis should represent a reasonable approximation of natural conditions.

The duration and concentration of exposures were also designed to reflect natural field concentrations based on practical and theoretical considerations. However, owing to the variable responses of each species numerous experiments using different dosages were necessary to invoke the desired plant stress level responses. The result was that a number of experiments showed little or no spectral response to exposure, whereas in other case the response was extreme. These upper and lower limits can be considered to reflect variability in gas levels spatially across a region, which would likely be encountered using an airborne platform. Note also that because of the time extensive nature of each experiment, duplicates of each experiment at a given dosage were not always possible. Thus, in some cases we were not able to verify, through duplication, spectral responses to some TICs.

The natural environment would also present a range of growth stages for a given species. It was shown in Phase I that there can be significant spectral variability with respect to growth stage. This variability would be inherent to the analysis of data acquired by an airborne platform. However, it is reasonable to expect that during an airborne acquisition of spectral data a particular growth stage (e.g. healthy mature) would dominate at a given time. The exception will be at times of the year such as spring and fall where plants species experience significant physiological changes (e.g. senescence).

We detected marked differences in physiological and spectral responses for some combinations of species and TICs. Different responses might be attributed in part to inherent differences in the structure and function of each species (e.g. stomatal density, cuticle thickness, leaf architecture, cell wall composition, growth and respiration rate), or the existence and efficiency of specific metabolic pathways for detoxifying TICs. Whatever their source, the existence of species-specific responses of vegetation to TICs presents both a challenge and an opportunity for regional remote sensing. In this study we were able to discern different spectral trends for NH_3, SO_2, Cl_2 and thus to distinguish these TICs. The trends result from the variable leaf response within plants, between plants and between species and it is expected much of the variability observed within species would be preserved or even enhanced in nature. As such it is encouraging for the possible detection of TIC effects on natural vegetation using airborne imagery. However the variability introduced from species to species that also enhances the TIC spectral trends would be a function of the landscape investigated and encompassed by a given data set (or geographical area) analyzed.

The larger goal was to develop a remote detection and monitoring capability for hazardous events such as a toxic gas leak. An ideal scenario would involve the release of the toxic chemicals investigated here leaked in the landscape. These agents cause stress and damage to surrounding vegetation the extent of which is dependent on dosage and time of exposure. Our findings at the leaf level suggest that damage can be detectable within 48hrs and should last for an extended period, probably up to a week and thus be possibly detectable from space depending on the repeat pass period of satellites (typically < 2 weeks). The detection tool developed and described in this study relies on the fact that damage is highly non uniform from leaf to leaf and plant to plant as one would expect in a field release thus successful detection in the natural landscape simply requires enough affected area (e.g. image pixels) to capture characteristic data trends for each TIC which should be feasible with <50 pixels (e.g. about 200mx200m for 30m pixels of planned HERO satellite). Such an area would encompass a wide spectrum of stress response from plants to TIC exposure and thus enable their detection. Because we developed a detection methodology from leaf observations there is an important knowledge gap that needs to be addressed using field trials to test if the findings of this study can extend to the detection of

leaks in the natural environment. The principal unknown is the effect of varying vegetation canopy structural parameters (e.g. canopy gaps, leaf area) and background properties (litter and soil reflectance) on the specific TIC data trends that were identified. Thus field trials should first be initiated with simulated TIC releases over closed and mature canopies (e.g. mature crops and conifer or broad leaf forests) since these conditions represent a natural extension of this leaf study. These trials could then be followed by releases over canopies over varied structural properties to test the robustness of the method.

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APPENDIX A

List of Experiments and Details

Date	Plant Species	no. CTR Plants	Details	Number Stressed Plants	Leaf Avg.	Averaged Library File Directory
Phase 1			**Growth Phases**			
7-Feb-06	poplar	4	4 stages of growth	N/A	yes	Phase1_poplar
8-Feb-06	poplar	19	4 stages of growth	N/A	yes	Phase1_poplar
9-Mar-06	wheat	25	3 stages of growth	N/A	yes	Phase1_wheat
10-Mar-06	wheat	21	3 stages of growth	N/A	yes	Phase1_wheat
6-Apr-06	canola	26	5 stages of growth	N/A	yes	Phase1_canola
Phase 2			**TIC Treatments**			
26-May-06	poplar	6	Na-Cl 500mM (cored)	7	yes	Phase2_poplar\Nacl\exp1
30-May-06	poplar	6	H20 ~ 24 hours	7	yes	Phase2_poplar\H2O\exp1
31-May-06	poplar	6	H20 ~ 48 hours	7	yes	Phase2_poplar\H2O\exp1
13-Jul-06	poplar	8	NH3 150-200ppm, 30-45min	5	yes	Phase2_poplar\NH3\exp1
			2 plants mock treated controls (2 plants exposed to <5min spike of <250ppm?) 2 mock plants in chamber, no gas used to test chamber specific effects (e.g. dehydration due to fan)	2 mock	yes	Phase2_poplar\NH3\exp1
			NaCl 150mM noon july 12th, 8 treated plants (soil drenched past saturation)	8	yes	Phase2_poplar\NaCl\exp2
14-Jul-06	poplar	8	NH3 150-200ppm, 30-45min	5	yes	Phase2_poplar\NH3\exp2
			2 plants mock treated controls (2 plants exposed to <5min spike of <250ppm?) 2 mock plants in chamber, no gas used to test chamber specific effects (e.g. dehydration due to fan)	2 mock	yes	Phase2_poplar\NH3\exp2
			NaCl 150mM noon july 12th, 8 treated plants (soil drenched past saturation)	8	yes	Phase2_poplar\NaCl\exp2
19-Jul-06	poplar	7	NaCl 150mM, re-applied to NaCl plants from July13/14	8	yes	Phase2_poplar\NaCl\exp3
			H2O noon july 12th	7	yes	Phase2_poplar\H2O\exp2
17-Aug-06	wheat	6	NH3 ~150ppm for 60 min, sampled 24hrs	5	yes	Phase2_wheat\NH3\exp1
			NaCl 200mM, sampled 24 hrs		yes	Phase2_wheat\NaCl\exp1
17-Aug-06	canola	8	NH3 ~150ppm for 60 min, sampled 24hrs	8	yes	Phase2_canola\NH3\exp1
18-Aug-06	wheat	6	NH3 ~150ppm for 60 min, sampled 48hrs	5	yes	Phase2_wheat\NH3\exp1

		NaCl 200mM, sampled 48 hrs	5	yes	Phase2_wheat\NaCl\exp1
18-Aug-06 canola	8	NH3 ~150ppm for 60 min, sampled 48hrs	8	yes	Phase2_canola\NH3\exp1
19-Oct-06 poplar	6	NH3 variable dosage (max 200 ppm), 24hrs	10	yes	Phase2_poplar\NH3\exp3
		NaCl 150mM. Sampled 24hrs	6	yes	Phase2_poplar\NaCl\exp4
20-Oct-06 poplar	6	NH3 variable dosage (max 200 ppm), 48hrs	10	yes	Phase2_poplar\NH3\exp3
		NaCl 150mM. Sampled 48hrs	6	yes	Phase2_poplar\NaCl\exp4
16-Nov-06 wheat	8	High dose ~200ppm NH3 for 30 min. 24hrs	9	yes	Phase2_wheat\NH3\exp3
		LOW dose ~50ppM NH3 for 30 min. 24hrs	8	yes	Phase2_wheat\NH3\exp2
17-Nov-06 wheat	8	HIGH dose ~200ppm NH3 for 30 min. 48hrs	9	yes	Phase2_wheat\NH3\exp3
		LOW dose ~50ppM NH3 for 30 min. 48hrs	8	yes	Phase2_wheat\NH3\exp2
7-Dec-06 canola	5	NaCl 150mM 24hrs	6	yes	Phase2_canola\NaCl\exp1
		NH3 low (~50ppm 20 min) 24hrs	6	yes	Phase2_canola\NH3\exp2
		NH3 high (~150-200ppm 30 min) 24hrs	5	yes	Phase2_canola\NH3\exp3
		Cl2 low (~3ppm 20 min) 24hrs	6	yes	Phase2_canola\Cl2\exp1
		Cl2 high (~15-20ppm 30 min) 24hrs	6	yes	Phase2_canola\Cl2\exp2
		H2S low (150ppm 30 min) 24hrs	6	yes	Phase2_canola\H2S\exp1
		H2S high (300ppm 30 min) 24hrs	7	yes	Phase2_canola\H2S\exp2
8-Dec-06 canola	5	NaCl 150mM 48hrs	6	yes	Phase2_canola\NaCl\exp1
		NH3 low (~50ppm 20 min) 48hrs	6	yes	Phase2_canola\NH3\exp2
		NH3 high (~150-200ppm 30 min) 48hrs	5	yes	Phase2_canola\NH3\exp3
		Cl2 low (~3ppm 20 min) 48hrs	6	yes	Phase2_canola\Cl2\exp1
		Cl2 high (~15-20ppm 30 min) 48hrs	6	yes	Phase2_canola\Cl2\exp2
		H2S low (150ppm 30 min) 48hrs	6	yes	Phase2_canola\H2S\exp1
		H2S high (300ppm 30 min) 48hrs	7	yes	Phase2_canola\H2S\exp2
7-Feb-07 canola	6	NaCl 150mM (cored) 24hrs	8	yes	Phase2_canola\NaCl\exp2
		SO2 low ~50ppm,	6	yes	Phase2_canola\SO2\exp1
		with a few spikes at ~100ppm 24hrs			
		SO2 high ~100ppm 24hrs	6	yes	Phase2_canola\SO2\exp2
		H2O 24hrs	5	yes	Phase2_canola\H2O\exp1
		NH3 ~150ppm (cored) 24hrs	8	yes	Phase2_canola\NH3\exp4
		Note: control are mock treated samples,			
		CTR cored			
8-Feb-07 canola	10	SO2 low ~50ppm,	6	yes	Phase2_canola\SO2\exp1
		with a few spikes at ~100ppm 48hrs			
		SO2 high ~100ppm 48hrs	6	yes	Phase2_canola\SO2\exp2
		H2O 48hrs	5	yes	Phase2_canola\H2O\exp1
		SO2 low <20ppm 30min 24hrs	6	yes	Phase2_canola\SO2\exp3
		NH3 <50ppm 30 min 24hrs	6	yes	Phase2_canola\NH3\exp5
		Note: 6 control are mock treated samples			
9-Feb-07 canola	4	NH3 low <50 ppm 30 min 48hrs	6	yes	Phase2_canola\NH3\exp5
		SO2 low < 20 ppm 30 min 48hrs	6	yes	Phase2_canola\SO2\exp3
13-Feb-07 canola		NH3 ~150ppm,	8	yes	Phase2_canola\NH3\exp4
		samples from Feb707 now showing stress			

27-Mar-07 canola	6	SO2 40 - > 100 ppm 24hrs only (HH ASD)	8	yes	Phase2_canola\SO2\exp4
		SO2 0 - 30 ppm 24 hrs (cored) (HH ASD)	6	yes	Phase2_canola\SO2\exp5
		3 ctr samples cored			
27-Mar-07 wheat	5	SO2 40 - > 100 ppm 24 hrs (HH ASD)	6	yes	Phase2_wheat\SO2\exp1
		SO2 0 - 30 ppm 24 hrs (HH ASD)	6	yes	Phase2_wheat\SO2\exp2
28-Mar-07 canola	4	Cl2 <5ppm 30 min 24hrs (HH ASD)	5	yes	Phase2_canola\Cl2\exp3
		Cl2 ~ 15ppm 30 min 24hrs (HH ASD)	5	yes	Phase2_canola\Cl2\exp4
28-Mar-07 wheat	5	SO2 40 - > 100 ppm 48 hrs (HH ASD)	6	yes	Phase2_wheat\SO2\exp1
		SO2 0 - 30 ppm 48 hrs (HH ASD)	6	yes	Phase2_wheat\SO2\exp2
		Cl2 <5ppm 30 min 24hrs (HH ASD)	5	yes	Phase2_wheat\Cl2\exp1
		Cl2 ~ 15ppm 30 min 24hrs (HH ASD)	5	yes	Phase2_wheat\Cl2\exp2
		NH3 ~ 140-200 ppm 24hrs (cored) (HH ASD)	6	yes	Phase2_wheat\NH3\exp4
		5 ctr samples cored			
29-Mar-07 canola	4	Cl2 <5ppm 30 min 48hrs (HH ASD)	5	yes	Phase2_canola\Cl2\exp3
		Cl2 ~ 15ppm 30 min 48hrs (HH ASD)	5	yes	Phase2_canola\Cl2\exp4
29-Mar-07 wheat	5	Cl2 <5ppm 30 min 48hrs (HH ASD)	5	yes	Phase2_wheat\Cl2\exp1
		Cl2 ~ 15ppm 30 min 48hrs (HH ASD)	5	yes	Phase2_wheat\Cl2\exp2
31-May-07 canola	6	SO2 low ~15-20ppm, 20min 24hrs	5	yes	Phase2_canola\SO2\exp6
		SO2 high ~75ppm, 20min 24hrs	6	yes	Phase2_canola\SO2\exp7
		Cl2 low ~2-3ppm, 20min 24hrs	6	yes	Phase2_canola\Cl2\exp5
		Cl2 high ~15-20ppm, 20min 24hrs	6	yes	Phase2_canola\Cl2\exp6
31-May-07 wheat	6	SO2 low ~15-20ppm, 20min 24hrs	6	yes	Phase2_wheat\SO2\exp3
		SO2 high ~75ppm, 20min 24hrs	6	yes	Phase2_wheat\SO2\exp4
		Cl2 low ~2-3ppm, 20min 24hrs	6	yes	Phase2_wheat\Cl2\exp3
		Cl2 high ~15-20ppm, 20min 24hrs	6	yes	Phase2_wheat\Cl2\exp4
1-Jun-07 canola	6	SO2 low ~15-20ppm, 20min 48hrs (cored)	5	yes	Phase2_canola\SO2\exp6
		SO2 high ~75ppm, 20min 48hrs (cored)	6	yes	Phase2_canola\SO2\exp7
		Cl2 low ~2-3ppm, 20min 48hrs (cored)	6	yes	Phase2_canola\Cl2\exp5
		Cl2 high ~15-20ppm, 20min 48hrs (cored)	6	yes	Phase2_canola\Cl2\exp6
		ctr samples cored			
1-Jun-07 wheat	6	SO2 low ~15-20ppm, 20min 48hrs	6	yes	Phase2_wheat\SO2\exp3
		SO2 high ~75ppm, 20min 48hrs (cored)	6	yes	Phase2_wheat\SO2\exp4
		Cl2 low ~2-3ppm, 20min 48hrs	6	yes	Phase2_wheat\Cl2\exp3
		Cl2 high ~15-20ppm, 20min 48hrs (cored)	6	yes	Phase2_wheat\Cl2\exp4
		ctr samples cored			
2-Oct-07 canola	6	Cl2 high 15-25ppm, 30 min 24hrs	6	yes	Phase2_canola\Cl2\exp7
		SO2 high 60-120 ppm, 40 min 24hrs	6	yes	Phase2_canola\SO2\exp8
		NaCl 400mM 24 hrs	8	yes	Phase2_canola\NaCl\exp3
		H2O 48hrs	6	yes	Phase2_canola\H2O\exp2
2-Oct-07 wheat	5	Cl2 high 15-25ppm, 30 min 24hrs	6	yes	Phase2_wheat\Cl2\exp5
		SO2 high 60-120 ppm, 40 min 24hrs	6	yes	Phase2_wheat\SO2\exp5

		NaCl 400mM 24 hrs	6	yes	Phase2_wheat\NaCl\exp2
		H2O 48hrs	6	yes	Phase2_wheat\H2O\exp1
3-Oct-07 canola	6	Cl2 high 15-25ppm, 30 min 48hrs	6	yes	Phase2_canola\Cl2\exp7
		SO2 high 60-120 ppm, 40 min 48hrs	4	yes	Phase2_canola\SO2\exp8
		NaCl 400mM 48hrs	8	yes	Phase2_canola\NaCl\exp3
		H2O 72hrs	6	yes	Phase2_canola\H2O\exp2
3-Oct-07 wheat	5	Cl2 high 15-25ppm, 30 min 48hrs	6	yes	Phase2_wheat\Cl2\exp5
		SO2 high 60-120 ppm, 40 min 48hrs	6	yes	Phase2_wheat\SO2\exp5
		NaCl 400mM 48hrs	6	yes	Phase2_wheat\NaCl\exp2
		H2O 72hrs	6	yes	Phase2_wheat\H2O\exp1
4-Oct-07 canola	6	HCN high >>50ppm, 30 min 24hrs	6	yes	Phase2_canola\HCN\exp1
		H2O 96hrs	6	yes	Phase2_canola\H2O\exp2
4-Oct-07 wheat	5	HCN high >>50ppm, 30 min 24hrs	3	yes	Phase2_wheat\HCN\exp1
		H2O 96hrs	6	yes	Phase2_wheat\H2O\exp1
5-Oct-07 canola	6	HCN high >>50ppm, 30 min 48hrs	6	yes	Phase2_canola\HCN\exp1
		H2O 120hrs	6	yes	Phase2_canola\H2O\exp2
5-Oct-07 wheat	5	HCN high >>50ppm, 30 min 48hrs	3	yes	Phase2_wheat\HCN\exp1
		H2O 120hrs	6	yes	Phase2_wheat\H2O\exp1

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This paper reports on the examination of the spectral response of individual leaves of three common Canadian plant species (poplar (Populus deltoides, Populus trichocarpa), wheat (Triticum aestivum), canola (Brassica napus)), which were subjected to fumigation with gaseous phase toxic industrial chemicals and chemicals precursor to chemical warfare agents (TICs), such as ammonia (NH3), sulphur dioxide (SO2), hydrogen sulphide (H2S), chlorine (Cl2), and hydrogen cyanide (HCN).

Analysis of the data using vegetation indices showed that the TICs and environmental stresses result in diagnostic light reflectance data trends from healthy mature to highly stressed leaves, with marked differences in physiological and spectral responses detected for some combinations of species and TICs. Further analysis showed that combinations of specific reflectance indices could be used to distinguish NH3, SO2, Cl2 consistently across all three species.

Findings at the leaf level suggest that both environmental stress and TIC treatments can be detectable within 48hrs and should last for an extended period, probably up to a week, and thus be possibly detectable from airborne/spaceborne imagery, depending on the overpass or observation period. However the variability introduced from species to species that also enhances the TIC spectral trends would be a function of the landscape investigated and encompassed by a given data set (or geographical area) analyzed. The next step should involve a field trial.

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